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INTER-ORGAN COMMUNICATION AND METABOLIC REGULATORS OF ADIPOSE TISSUE AND SKELETAL MUSCLE

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Cover illustration: The cover art illustrates inter-organ and cell-cell communication. As dominos fall, they depict a cascade of signals, with some dots traveling from one domino to the next, symbolizing signal propagation. The varying shades and clustering of dots reflect signal strength and amplification. The house of dominos represents structure within the system, but remains susceptible to destabilization by stressors (e.g., disease or exercise), which can disrupt signal flow and impact physiological outcomes. Conceptualized by Kyle Dumont and created by António Neves.

Inter-Organ Communication and Metabolic Regulators of Adipose Tissue and Skeletal Muscle

Thesis for Doctoral Degree (Ph.D.)

By

Kyle D. Dumont

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To Grandma & Grandpa Bell—

Who I am is a reflection of the love, kindness, and respect you lived by.

I miss you every day.

Abstract

Inter-organ communication is essential for maintaining homeostasis, enabling different organ systems to coordinate and adapt to physiological demands. Disruptions in these communication networks can lead to metabolic dysfunction and disease. This thesis investigates the role of inter-organ communication in regulating metabolic processes, with a focus on adipose tissue and skeletal muscle. Additionally, we examine how specific signaling molecules—particularly those of the kynurenine pathway of tryptophan degradation—interact with these tissues to influence metabolic health and disease. We explore how these organs communicate in response to stressors and how this communication becomes disrupted in disease.

In **Paper I**, we investigate how changes in circulating kynurenine metabolites affect metabolism. We show that despite marked changes in circulating kynurenine metabolites, whole-body energy metabolism remains largely unaffected. Importantly, we highlight how the metabolic benefits of kynurenic acid depend on its intermittent increase in circulation, similar to transient exercise-induced signals that mediate improved metabolic health.

In **Paper II**, we identify the gene *Zfp697/ZNF697* as a novel regulator of muscle regeneration, uncovering its crucial role in skeletal muscle recovery following injury.

In **Paper III**, we demonstrate that sensory neuron-derived alpha-calcitonin gene-related peptide (CGRP α) regulates key factors of adipose tissue, including adipogenesis, extracellular matrix remodeling, and adipocyte size distribution.

Together, these studies enhance our understanding of how inter-organ communication influences health and disease, offering exciting therapeutic opportunities for improving metabolic health.

List of scientific papers

- I. **Constitutive loss of kynurenine-3-monooxygenase changes circulating kynurenine metabolites without affecting systemic energy metabolism**

Kyle D. Dumont, Paulo R. Jannig, Margareta Porsmyr-Palmertz, Jorge L. Ruas. *Am J Physiol Endocrinol Metab.* 2025 Feb 1;328(2):E274–E285. doi: 10.1152/ajpendo.00386.2024. Epub 2025 Jan 13. PMID: 39805032.

- II. **Zfp697 is an RNA-binding protein that regulates skeletal muscle inflammation and remodeling**

Jorge C. Correia, Paulo R. Jannig, Maya L. Gosztyla, Igor Cervenka, Serge Ducommun, Stine M. Præstholt, José M. Dias, **Kyle D. Dumont**, Zhengye Liu, Qishan Liang, Daniel Edsgård, Olof Emanuelsson, Paul Gregorevic, Håkan Westerblad, Tomas Venckunas, Marius Brazaitis, Sigita Kamandulis, Johanna T. Lanner, Ana I. Teixeira, Gene W. Yeo, Jorge L. Ruas. *Proc. Natl. Acad. Sci. U.S.A.* 2024 Aug 20;121(34):e2319724121. doi: 10.1073/pnas.2319724121. PMID: 39141348.

- III. **Sensory neuron-derived α -calcitonin gene-related peptide controls adipogenesis**

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List of abbreviations

3-HK	3-hydroxykynurenine
AA	anthranilic acid
AAV	adeno-associated virus
AC	Adenylate cyclase
AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
ATGL	adipose triglyceride lipase
ATP	adenosine triphosphate
BAT	brown adipose tissue
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BMP	bone morphogenetic protein
BNIP3	BCL2 interacting protein 3
C/EBP	CCAAT-enhancer-binding protein
cAMP	Cyclic adenosine monophosphate
CDK	cyclin-dependent kinases
CGRP α	alpha-calcitonin gene-related peptide
CNS	central nervous system
CRISPR	clustered regularly interspaced short palindromic repeats
DAMP	damage-associated molecular patterns
DMD	Duchenne muscular dystrophy
DMDF	damaged myofiber-derived factors
DNA	deoxyribonucleic acid
ECM	extracellular matrix
ERK	extracellular signal-regulated kinase
FA	fatty acid
FAP	fibroadipogenic precursor
FFA	free fatty acid
FGF	fibroblast growth factor
FOXO	forkhead box transcription factor
FoxO3	forkhead box O3
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GLP-1	glucagon-like peptide-1
GPR35	G-protein coupled receptor 35
HFD	high-fat diet
HGF	hepatocyte growth factor
HSL	hormone sensitive lipase
IDO	indoleamine 2,3-dioxygenase
IFN- γ	interferon-gamma
IGF-1	insulin-like growth factor-1
IKK	inhibitory- κ B kinase
IL	interleukin
JAK	janus kinase
KAT	kynurenine aminotransferase

KMO	kynurenine-3-monooxygenase
KYNA	kynurenic acid
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MCE	mitotic clonal expansion
MGL	monoglyceride lipase
MMP	matrix metalloproteinase
MRF	myogenic regulatory factor
MSC	mesenchymal stem cell
mTOR	mammalian target of rapamycin
mTORC1	mTOR complex 1
MuRF1	muscle RING finger 1
Myf5	myogenic factor 5
NAD ⁺	nicotinamide adenine dinucleotide
NE	norepinephrine
NF- κ B	nuclear factor-kappa B
NMDA	N-methyl-D-aspartate
NMDAR	NMDA receptor
NMJ	neuromuscular junction
Nnt	nucleotide transhydrogenase
NO	nitric oxide
NOS	nitric oxide synthase
PGC-1 α	PPAR γ coactivator-1 α
PKA	protein kinase A
PLIN	perilipin
PPAR γ	peroxisome proliferator-activated receptor γ
QUIN	quinolinic acid
RNA	ribonucleic acid
ROS	reactive oxygen species
SNS	sympathetic nervous system
SP	substance P
STAT	signal transducer and activator of transcription
SVF	stromal vascular fraction
TDO	tryptophan-2,3-dioxygenase
TG	triglyceride
TGF	transforming growth factor
TRPV1	transient receptor potential vanilloid-1
UCP1	uncoupling protein 1
UPS	ubiquitin-proteasome system
VAT	visceral adipose tissue
VEGF	vascular endothelial growth factor
WAT	white adipose tissue
WNT	wingless-related integration site
Zfp697	zinc finger protein 697
β -AR	β -adrenergic receptor

1 Introduction

The survival and adaptability of multicellular organisms depend on the seamless coordination of physiological systems. This coordination is mediated by complex networks of inter-organ communication that integrate internal and external signals to maintain homeostasis. These networks operate through the central and peripheral nervous systems, as well as the exchange of signaling molecules, including hormones, metabolites, and cytokines. Together, they enable the organism to respond dynamically to fluctuations in nutrient availability, energy demand, and environmental stressors.

Maintaining homeostasis requires that inter-organ communication remains intact and functionally effective. When these systems falter, they undermine the organism's ability to defend homeostasis, leading to widespread effects on health, such as growth, development, immunity, and metabolism. Understanding how organs communicate and regulate each other's activity is therefore essential for uncovering the mechanisms underlying health and disease.

The work summarized in this thesis investigates fundamental aspects of inter-organ communication, with a particular focus on adipose tissue and skeletal muscle—two tissues that undergo remodeling in response to stressors and are central to systemic energy metabolism. By exploring the molecular and cellular pathways that link these tissues to other organs, this work aims to uncover how their involvement in the broader communication network influences systemic physiology, tissue remodeling, and metabolic regulation. These findings will provide insights into how the body coordinates its responses to maintain homeostasis and how disruptions in these processes may contribute to disease.

1.1 Inter-Organ Communication

Inter-organ communication is a fundamental physiological process through which organs exchange signals to coordinate responses and regulate systemic homeostasis. This dynamic signaling ensures that organs respond appropriately to internal and external stimuli, such as metabolic needs or environmental stressors, and orchestrate functions like metabolism, immune responses, and nutrient allocation. As our understanding of these communication networks evolves, we gain valuable insights into their roles in both health and disease.

1.1.1 Historical Foundations of Inter-Organ Communication

The importance of inter-organ communication has been recognized for centuries, with early contributions shaping our understanding of how organs coordinate their activity. One of the first recorded ideas of communication between organs comes from the work of the Greek physician Galen of Pergamon in the 2nd century, who recognized that the body is unified not by distinct boundaries between its parts, but by the interconnectedness of its various components [1]. However, it wasn't until the 19th century that the concept of physiological regulation through inter-organ signaling gained substantial recognition. Claude Bernard, often referred to as the father of modern physiology, introduced the idea of the "milieu intérieur"—the internal environment of the body that must remain stable despite external changes [2]. This concept laid the foundation for understanding how organs interact and maintain physiological balance. Building on Bernard's ideas, Walter Cannon coined the term "homeostasis" in the early 20th century, emphasizing how multiple organs interact to maintain internal stability in response to physiological stressors [3, 4]. These foundational ideas established a framework for understanding the role of inter-organ communication in maintaining physiological balance.

Since the pioneering work of Bernard and Cannon, our understanding of inter-organ communication has evolved significantly. Disturbances in this signaling network have been implicated in a range of diseases, including metabolic disorders [5, 6], aging-related pathologies [7], and mental health conditions [8]. These insights emphasize the crucial role of coordinated inter-organ communication in maintaining health [9]. As our understanding deepens, it has become increasingly clear that the mechanisms driving inter-organ communication are complex and multifaceted. This growing body of research

now focuses on the molecular players and signaling pathways that coordinate these physiological interactions, offering new insights into how organs communicate at the cellular and molecular levels.

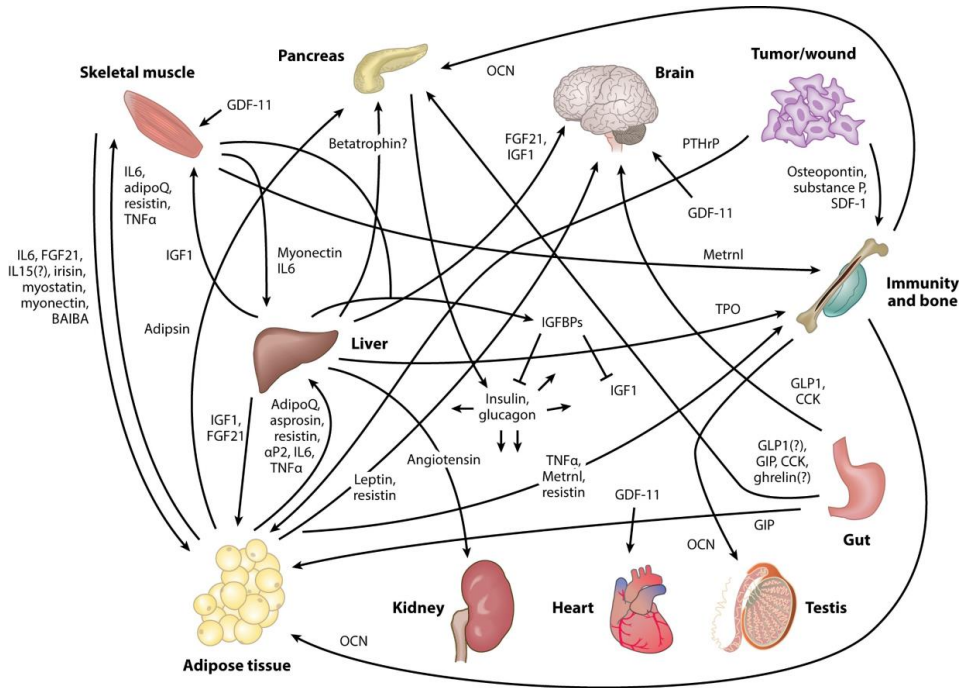
1.1.2 The Inner Dialogue: Mechanisms of Inter-Organ Communication

To fully grasp how organs communicate, it is essential to first understand the underlying mechanisms of this intricate dialogue. The mechanisms of inter-organ communication are as diverse as the physiological processes they regulate. In theory, any molecule that cells can sense could serve as a signaling tool, offering limitless possibilities for tailoring messages and achieving precise cellular responses. At the heart of this complexity lies the cell—the fundamental unit of tissues and organs—that both sends and receives these signals.

Cells communicate through three primary modes: endocrine, paracrine, and autocrine signaling. Endocrine signaling operates over long distances, traveling through the bloodstream to influence target tissues. A classic example of endocrine signaling is insulin, a peptide hormone secreted by the pancreas which regulates glucose uptake in distant tissues like skeletal muscle and adipose tissue. Paracrine signaling, by contrast, occurs between neighboring cells within a tissue; for instance, immune cells release cytokines to recruit and activate neighboring cells during tissue repair or inflammation. Meanwhile, in autocrine signaling, cells respond to signals they themselves produce, such as transforming growth factor- β (TGF- β), which regulates cell proliferation and differentiation [10]. Together, these signaling modes play unique and complementary roles, forming a dynamic and adaptable network that ensures physiological coordination across the organism.

The molecular "language" of these communication modes is remarkably versatile, including peptides (e.g., insulin, leptin), metabolites (e.g., lactate, bile acids), lipids (e.g., prostaglandins), and neurotransmitters (e.g., norepinephrine). Each molecule is tailored to carry specific messages and is regulated in response to distinct cues, creating a dynamic system to regulate physiological responses (Figure 1.1). For example, when blood glucose levels rise after eating, the pancreas secretes insulin, promoting glucose uptake into cells and lowering blood sugar levels. Conversely, when blood glucose levels drop, the pancreas releases glucagon, signaling the liver to release stored glucose and raise blood sugar levels. This feedback loop highlights how signaling molecules like insulin and

glucagon mediate the body's response to changes in the internal environment, ultimately regulating blood sugar levels.



AR Droujinine IA, Perrimon N. 2016.
Annu. Rev. Genet. 50:539–70

Figure 1.1. Inter-organ communication: Signaling factors and their origins

Examples of mammalian inter-organ communication factors, their tissue origins, and destinations, emphasizing the complexity of communication between organs. Reproduced with permission from the Annual Review of Genetics, Volume 50 © 2016 by Annual Reviews, <http://www.annualreviews.org> [11].

Beyond these chemical messengers, other pathways expand the "vocabulary" of inter-organ communication. The nervous system, with its complex network of neurons, provides rapid and precise signaling between organs, complementing slower, more sustained endocrine signals. Additionally, extracellular vesicles—small, membrane-bound particles—have emerged as versatile carriers of bioactive molecules [12]. These alternative pathways enhance the organism's ability to coordinate and regulate physiological responses, adding layers of precision and flexibility to the system.

Given the plethora of molecules secreted by these organs, each organ has a specific class of signaling factors dedicated to it. These classes are named with a suffix "-kine," derived from the Greek word "kinesis," meaning movement,

combined with a prefix that indicates the originating organ. For example, adipose tissue releases adipokines (adipo = adipose), skeletal muscle releases myokines (myo = muscle), and the liver releases hepatokines (hepato = hepatocyte, the main cell type of the liver). This naming convention underscores the significance of these secreted factors, as it reflects the attention given to their roles in inter-organ communication.

By leveraging these diverse signaling modes and molecular tools, cells within tissues—and the organs they comprise—collectively form a sophisticated communication network. Whether acting locally or systemically, these mechanisms enable the body to dynamically regulate metabolism, immune responses, and tissue repair, ensuring a constant dialogue that facilitates adaptive physiological responses.

1.1.3 The Central Role of Inter-Organ Communication in Metabolic Health

The inter-organ dialogue is not merely an intricate signaling system—it is the foundation upon which whole-body metabolic health depends [5, 6, 13]. Maintaining metabolic health requires a continuous and coordinated exchange of information among organs to ensure sufficient energy availability. This coordination is particularly critical during metabolic stressors, such as extreme feeding patterns and physical activity.

Central to metabolic health is the interplay between key metabolic organs such as the pancreas, liver, adipose tissue, skeletal muscle, and the brain, each with a specialized role in metabolic regulation. These organs work together to maintain energy homeostasis, ensuring that energy is distributed appropriately according to physiological needs. For example, adipose tissue releases hormones such as leptin and adiponectin, which regulate appetite, glucose metabolism, and energy expenditure [14]. Similarly, skeletal muscle releases signaling molecules like interleukin-6 (IL-6), which influences glucose metabolism and energy homeostasis, particularly in response to exercise [15]. The liver plays a crucial role in sensing nutrient availability and regulating metabolic processes, acting as a central mediator during feeding and fasting transitions by shifting from energy storage to energy utilization [16]. The brain integrates a wide range of sensory and metabolic signals, processing signals and detecting circulating metabolic hormones and nutrients to maintain metabolic homeostasis [17, 18].

In pathological metabolic conditions like obesity, however, disruptions in inter-organ communication can not only contribute to the development of these

disorders but also exacerbate pre-existing metabolic imbalances. For example, in obesity, excessive food intake leads to increased secretion of insulin from the pancreas. Normally, this insulin would promote the storage of excess energy in adipose tissue. However, chronic overproduction of insulin leads to the adipose tissue becoming insulin resistant [19]. As a result, excess energy is diverted to other tissues, such as the liver and skeletal muscle, where it further exacerbates the underlying metabolic pathology. Moreover, this dysregulated signaling is often compounded by low-grade inflammation [19], which further impairs insulin action and tissue function, contributing to a cycle of metabolic pathology.

In the following sections, we will explore the roles of adipose tissue and skeletal muscle in inter-organ communication. We will examine how these tissues maintain energy balance, support tissue health, and regulate metabolism. Additionally, we will discuss how specific signaling molecules interact with adipose tissue and skeletal muscle, influencing overall metabolic health. Understanding the contributions of adipose tissue, skeletal muscle, and related metabolic pathways to systemic metabolism will provide valuable insights into how targeting these networks could offer novel therapeutic strategies to improve both metabolic health and tissue function.

1.2 Adipose Tissue

Adipose tissue is a vital component of the body's metabolic network, playing a fundamental role in energy storage, energy availability, and overall metabolic health. Though often associated with energy storage, adipose tissue is a complex and dynamic organ with diverse functions. In this section, we will discuss key aspects of adipose tissue, including its physiological roles in health, its different functional forms, and the processes involved in its growth and expansion. Additionally, we will discuss how disruptions in adipose tissue function contributes to metabolic diseases and how its regulation is intertwined with other organs in the body's metabolic network. By the end of this section, we will have established a comprehensive understanding of adipose tissue's pivotal role in both health and disease.

1.2.1 Adipose Tissue in Health

The evolving understanding of adipose tissue highlights its integral role in metabolic regulation, not merely as an energy store, but as a key player in inter-organ signaling and systemic health. Historically, adipose tissue was seen as a passive reservoir for fat, a concept rooted in early anatomical studies. This view persisted until the discovery of leptin in the 1990s [20], which dramatically shifted the scientific community's perspective. Leptin, a hormone secreted by adipocytes, was found to regulate appetite and energy expenditure by signaling to the brain [21]. This discovery opened the door to understanding the broader metabolic roles of adipose tissue, leading to the realization that adipose is not just a storage site, but a dynamic endocrine organ.

Building on this, the functional complexity of adipose tissue becomes even more apparent when we explore its cellular composition. Adipose tissue is made up of a variety of specialized cell types, including adipocytes, preadipocytes, immune cells, endothelial cells, and fibroblasts, each playing distinct yet interconnected roles in tissue function and metabolism [22]. While adipocytes are most closely associated with adipose tissue, the function of this tissue depends on a coordinated effort between these diverse cell types. Preadipocytes serve as progenitor cells, capable of differentiating into adipocytes to meet the tissue's expanding needs. Immune cells regulate local inflammation, playing a crucial role in tissue remodeling and response to metabolic stress. Endothelial cells form blood vessels that deliver nutrients and oxygen, essential for tissue health and function. Fibroblasts, producing the extracellular matrix, provide structural

integrity and facilitate the tissue's dynamic adaptability. Together, these diverse cell types enable adipose tissue to serve not only as an energy reservoir but to also actively participate in systemic metabolic regulation and endocrine signaling [22].

1.2.2 The Different Colors of Adipose Tissue

Adipose tissue displays a range of colors that correspond to distinct cell types and depot characteristics. The primary adipocyte types—white (from white adipose tissue or WAT), brown (from brown adipose tissue or BAT), beige (or brite, i.e., brown-in-white), and pink—each have unique anatomical and functional properties (Figure 1.2). Although all adipocytes store lipids, their differing hues reflect variations in function and metabolic activity.

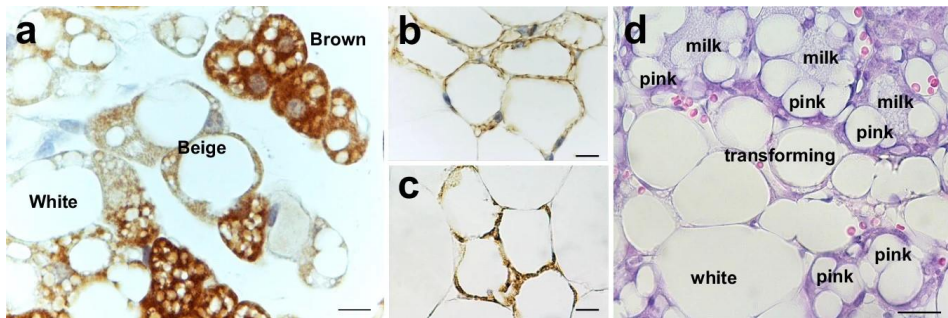


Figure 1.2. Adipocyte diversity: White, brown, beige, and pink adipocytes

(A) UCP1 staining in perirenal adipose tissue of a cold-exposed mouse shows unilocular, UCP1-negative adipocytes (white), multilocular UCP1-positive adipocytes (brown), and several intermediate forms showing weak UCP1 (beige). (B) Subcutaneous adipose tissue of a mouse after 2 hours at 4°C reveals partially multilocular UCP1-positive adipocytes. (C) Perirenal adipose tissue from a 53-year-old man in Siberia resembles cold-exposed adipocytes in (B). (D) Mammary gland of a pregnant mouse displays white adipocytes, transforming forms, and epithelial cells with lipid droplets (pink adipocytes). Scale bars: 10 μ m (A, B, D); 35 μ m (C). Reprinted with permission [23–25].

WAT is the most abundant adipose tissue in the adult human body and is composed primarily of white adipocytes under healthy conditions. These cells contain a single, large, unilocular lipid droplet that displaces organelles such as mitochondria and the nucleus to the cell periphery (Figure 1.2A) [26, 27]. The high lipid content gives WAT its characteristic pale or yellowish appearance. WAT's primary role is energy storage—accumulating triglycerides for later use when energy demands increase. In addition, WAT functions as an endocrine organ, secreting hormones like leptin and adiponectin to regulate metabolism, energy balance, and insulin sensitivity [28]. In disease states, WAT often exhibits

increased immune cell infiltration, particularly by macrophages, leading to a pro-inflammatory environment. This shift is associated with metabolic dysfunction, as chronic inflammation can impair adipocyte function, disrupt insulin signaling, and contribute to conditions such as obesity and type 2 diabetes [28, 29].

In contrast, BAT has a reddish-brown color due to its rich mitochondrial content containing heme cofactors in the cytochrome oxidase enzyme (Figure 1.2 A-C) [30]. Unlike white adipocytes, which contain a single large, unilocular lipid droplet, brown adipocytes contain multiple smaller lipid droplets. These cells also express high levels of genes involved in oxidative phosphorylation, particularly uncoupling protein 1 (UCP1) [30]. UCP1 plays a central role in converting chemical energy directly into heat—a process known as non-shivering thermogenesis [27]. This thermogenic capacity is essential for maintaining body temperature in newborns and hibernating animals [31]. Given its ability to dissipate energy as heat, activating BAT has emerged as a potential strategy for combating obesity and promoting metabolic health by increasing energy expenditure [32, 33].

Beige, or brite (brown-in-white), adipocytes are a result of the process known as beiging or browning, in which white adipocytes take on features of brown adipocytes in response to stimuli such as cold exposure or sympathetic nervous system activation (Figure 1.2A-C) [26]. Like brown adipocytes, beige adipocytes contain multiple smaller lipid droplets, but they typically lack the high mitochondrial content and oxidative phosphorylation markers characteristic of classical brown fat. During beiging, white adipocytes upregulate UCP1 and engage in non-shivering thermogenesis, converting chemical energy into heat. Once the stimuli are removed, beige adipocytes undergo mitophagy-mediated mitochondrial removal, reverting to a phenotype that resembles white adipose tissue, thus restoring the characteristics of white fat [34]. Notably, beige adipocytes do not form a distinct fat depot; instead, they are interspersed throughout WAT, contributing to a depot's overall beige phenotype under appropriate conditions. Given their ability to dissipate energy as heat like BAT, efforts to therapeutically enhance the beiging of white adipocytes are being explored as a strategy to increase energy expenditure and improve metabolic health, with potential applications for obesity treatment [35].

The final adipocyte type discussed here is the pink adipocyte. During pregnancy and lactation, subcutaneous adipose depots transform into mammary glands, where lipid-rich epithelial cells, known as pink adipocytes, are formed (Figure

1.2C) [36]. Although the precise origin of pink adipocytes is still uncertain, one hypothesis suggests that they arise from the transdifferentiation of white adipocytes within the mammary gland during pregnancy and lactation [36]. During this process, white adipocytes transform into milk-secreting epithelial cells, termed pink adipocytes, acquiring characteristics necessary for lactation. Unlike white, brown, and beige adipocytes, which play key roles in energy storage and expenditure, pink adipocytes play a specialized role in milk production and contribute to the development and maturation of the mammary duct system during lactation [26]. After lactation, these cells can revert to their original adipocyte state, demonstrating remarkable cellular plasticity.

The diverse colors of adipose tissue reflect its functional and metabolic heterogeneity, positioning it as a central focus in the study of energy metabolism. Research on the various adipocyte types—ranging from the energy-storing white adipocytes to the thermogenically active brown and beige adipocytes, and further to the specialized pink adipocytes involved in lactation—is deepening our understanding of their unique physiological roles and contributions to health and disease. Moreover, the dynamic interplay and transformation between these adipocyte types present a compelling area of study, with the potential to inspire novel therapeutic strategies for improving metabolic health and energy balance.

1.2.3 The Anatomical Diversity of Adipose Tissue Depots

Adipose tissue is not only functionally diverse, but its anatomical distribution also plays a key role in determining its distinct physiological functions [37–40]. While the most common classification scheme divides adipose tissue into subcutaneous and visceral depots, this oversimplification overlooks the complexity within these categories. Within these broad categories, adipose depots can be further subdivided, with each depot exhibiting distinct functional differences. Given the focus of this thesis, the following section will focus on two well-characterized and widely studied WAT depots in mouse models: inguinal white adipose tissue (iWAT), representing subcutaneous fat, and gonadal white adipose tissue (gWAT, sometimes referred to as epididymal white adipose tissue or eWAT), representing visceral fat (Figure 1.3). This section will briefly highlight their correlation to human adipose depots, physiological roles, relevance to metabolic health.

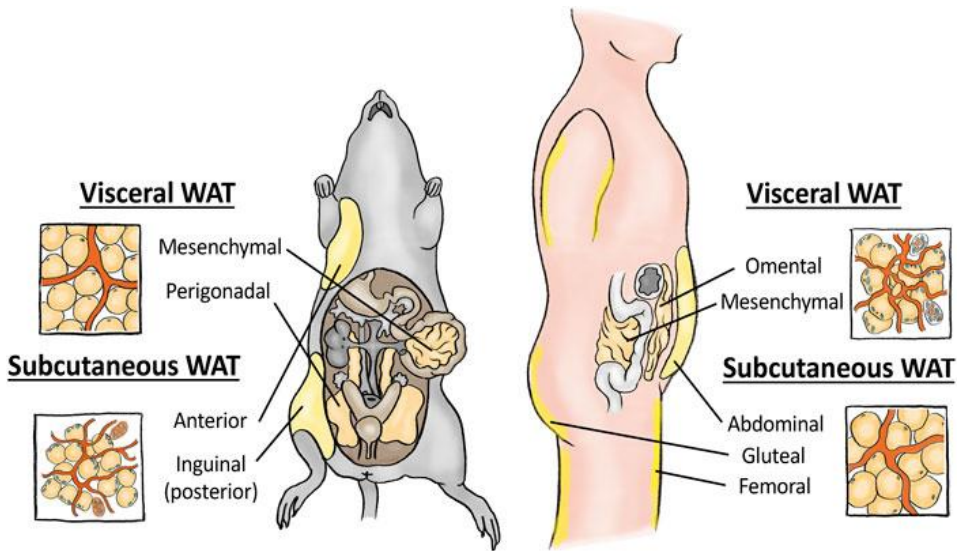


Figure 1.3. Visceral and subcutaneous adipose tissue depots in mice and humans

Schematic representation of the major visceral (gonadal; gWAT) and subcutaneous (inguinal; iWAT) white adipose tissue depots in mice (left) and humans (right). Reprinted under the Creative Commons Attribution License (CC BY) [41].

In mouse models, iWAT and gWAT are the primary depots used to study subcutaneous and visceral fat, respectively. iWAT is in the inguinal region, just beneath the skin, whereas gWAT is found adjacent to the gonads within the abdominal cavity (Figure 1.3) [41]. iWAT serves as a reasonable model for human subcutaneous adipose tissue due to similarities in location and metabolic function [22]. However, gWAT lacks a direct counterpart in humans, as the distribution of visceral adipose tissue in humans is markedly different, with much of it localized to the omentum, a structure that is only minimally present in rodents [27]. These distinctions underscore the importance of considering species-specific differences when interpreting findings from mouse adipose tissue studies.

In humans, subcutaneous and visceral adipose tissues exhibit distinct metabolic properties with divergent implications for health [42]. Subcutaneous fat (iWAT), for example, is associated with a lower risk of metabolic disorders such as type 2 diabetes and cardiovascular disease. In contrast, visceral (gWAT) is linked to adverse metabolic outcomes [42]. Two main hypotheses attempt to explain this disparity: (1) depot-specific innervation and blood flow, where visceral fat drains into the portal circulation, directly influencing the liver, or (2) intrinsic differences in adipocyte function driven by cell-autonomous mechanisms [27]. While these

two hypotheses are not mutually exclusive, evidence supporting the latter has solid ground.

For example, preadipocytes show depot-specific gene expression and maintain unique characteristics even when cultured under the same conditions [43–46]. Correspondingly, the expression of adipogenic transcription factors like PPAR γ and C/EBP α , which regulate adipocyte differentiation, lipid storage, and the secretion of hormones and cytokines, is more abundant in subcutaneous fat [43, 47]. This suggests that subcutaneous adipocytes are more efficient in storing fat and secreting beneficial adipokines compared to their visceral counterparts [40]. In contrast, reduced expression of these transcription factors in visceral adipose tissue (VAT) may limit its ability to adapt to metabolic demands and increase its susceptibility to dysfunction.

These molecular differences are further reflected in the plasticity of adipose tissue depots following environmental stressors. A remarkable characteristic of adipose tissue is its ability to dramatically alter its size through the enlargement of individual cells (hypertrophy) or the recruitment of new adipocytes (hyperplasia), or both (Figure 1.4). In response to high-fat feeding and cold exposure in mice, iWAT and gWAT exhibit distinct remodeling patterns [48, 49]. In high-fat feeding conditions, iWAT primarily undergoes hypertrophy, whereas gWAT shows both hypertrophy and hyperplasia. Following cold exposure, iWAT recruits new beige adipocytes, while gWAT recruits new white adipocytes [48]. Importantly, these data highlight depot-specific differences, but they should not be viewed as absolutes, as they can vary across different experimental models [49].

Depot-specific differences are also evident in the secretion of key factors [40]. Leptin, a hormone beneficial for metabolic health, is expressed at higher levels in subcutaneous fat compared to visceral fat [43]. Furthermore, inflammation plays a significant role in shaping depot characteristics and is involved in regulating the healthy expansion of adipose tissue [50]. Pro-inflammatory cytokines, such as TNF- α and IL-6, are produced by adipocytes and influence metabolic health. For instance, TNF- α inhibits the breakdown of triglycerides to fatty acids and glycerol by lipoprotein lipase and suppresses the expression of key adipogenic transcription factors, thereby impairing lipid metabolism and promoting insulin resistance [51, 52]. Both TNF- α and IL-6 are produced at higher levels in visceral fat, contributing to local inflammation, adipocyte apoptosis, and insulin

resistance [53–55]. Although VAT is often characterized by a pro-inflammatory profile, this is not an intrinsic property of the depot itself but rather a reflection of metabolic and physiological conditions. In aged lean mice for example, VAT exhibits an anti-inflammatory and highly lipolytic phenotype, a state that can be further enhanced by endurance exercise, underscoring the depot's dynamic and adaptive nature [56].

These molecular differences underscore the functional diversity of adipose tissue depots and their significant impact on metabolic health. The depot-specific characteristics—from hormone secretion to inflammatory responses—help explain the contrasting metabolic effects of subcutaneous and visceral fat. Subcutaneous fat, with its higher leptin levels and healthier inflammatory profile, contributes to improved insulin sensitivity and energy balance regulation. In contrast, the inflammatory environment of visceral adipose tissue and its lower leptin production are associated with metabolic disturbances, such as insulin resistance and a heightened risk of metabolic diseases [42]. Overall, the anatomical distribution of adipose tissue is a critical factor to consider when studying its role in health and disease.

1.2.4 Adipogenesis and Adipose Tissue Expansion

Adipose tissue serves as the primary energy reservoir in the body, storing and releasing energy in response to hormonal signals and energetic demands. The ability of mature adipocytes to sequester lipids is crucial for protecting tissues vulnerable to lipotoxicity, such as the liver, muscle, and heart. Defects in *de novo* adipogenesis—the formation of new adipocytes from progenitor cells—can limit the lipid storage capacity of adipose tissue, leading to improper lipid handling and precipitating obesity-related metabolic disorders. Furthermore, the metabolically favorable expansion of adipose tissue depends on the recruitment and differentiation of adipose progenitor cells (Figure 1.4) [57, 58]. Notably, humans retain functional adipose progenitors throughout adulthood, capable of differentiation and contributing to tissue remodeling [59]. Processes that affect adipogenesis may therefore provide critical insights into the pathophysiology of metabolic disorders and reveal potential therapeutic targets.

Adipogenesis is a highly organized and multifaceted process through which precursor cells commit to and mature into functional adipocytes (Figure 1.5). This process is orchestrated by an intricate network of signaling pathways and transcription factors that regulate the expression of genes necessary for

adipocyte development [60–65]. Within the stromal vascular fraction (SVF) of adipose tissue lies a pool of pluripotent mesenchymal stem cells (MSCs), which serve as the progenitors for adipocytes. Due to their pluripotency, MSCs can differentiate into multiple cell types, including myocytes, chondrocytes, osteocytes, and adipocytes (Figure 1.5) [66, 67].

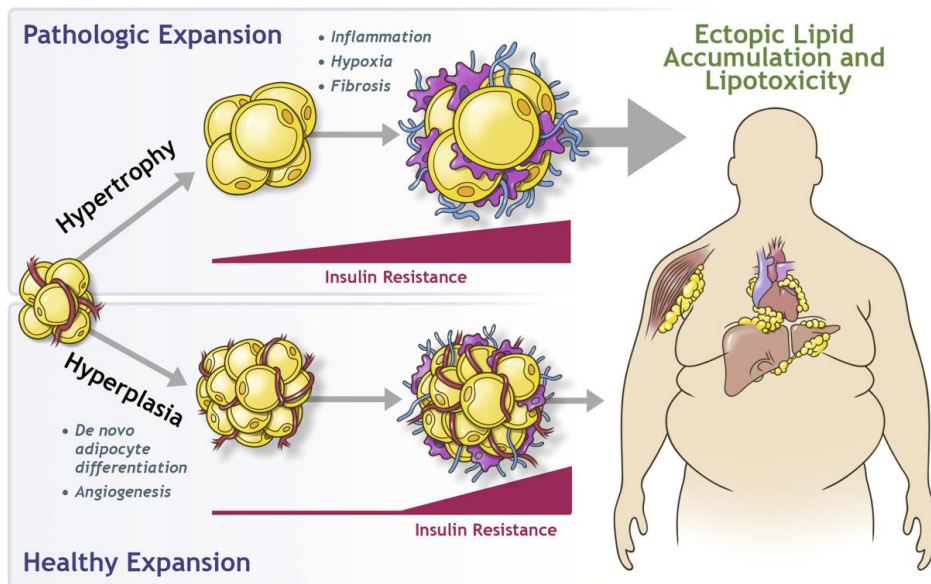


Figure 1.4. White adipose tissue expansion in obesity

In response to caloric excess, white adipose tissue expands through two mechanisms: hypertrophy (enlargement of existing adipocytes, top) and hyperplasia (increase in adipocyte number, bottom). Pathological expansion, characterized by hypertrophy, is associated with inflammation, fibrosis, and the early onset of insulin resistance. In contrast, healthy expansion occurs through hyperplasia, involving the recruitment and differentiation of adipose progenitor cells, along with angiogenesis. This process helps maintain proper lipid storage and prevents ectopic lipid accumulation, delaying insulin resistance. Reprinted with permission [68].

The process of adipogenesis can be divided into distinct phases. The initial commitment phase involves lineage specification, during which MSCs transition into preadipocytes and lose their pluripotent characteristics. This is followed by terminal differentiation, wherein preadipocytes acquire the defining features of mature adipocytes (Figure 1.5). A transient but critical phase of cell proliferation, known as mitotic clonal expansion, is also required to ensure the proper development of these cells [69]. At the molecular level, adipogenesis is governed by a network of transcription factors, with the CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptor γ (PPAR γ) playing

central roles in orchestrating the expression of genes necessary for adipocyte development.

In the following sections, we delve into these interconnected phases of adipogenesis, highlighting the cellular transitions, signaling events, and transcriptional regulation that underpin this process. Particular focus will be placed on the pivotal roles of C/EBPs and PPAR γ , as well as the mechanisms driving mitotic clonal expansion and their coordination with commitment and terminal differentiation. Together, these discussions aim to provide a comprehensive understanding of the molecular and cellular events that govern adipocyte formation.

1.2.4.1 *Commitment*

The commitment of MSCs to the adipocyte lineage is a crucial early step in adipogenesis and is regulated by a variety of signaling pathways. Two major families of signaling molecules—WNT/ β -catenin and transforming growth factor- β (TGF- β)—play central roles in this process.

The WNT family of glycoproteins is key to the regulation of MSC fate. WNT proteins are secreted to the extracellular matrix and can activate receptor-mediated signaling in nearby cells, influencing diverse cellular functions such as proliferation, apoptosis, and differentiation [67, 70, 71]. Notably, the WNT signaling pathway acts as a critical determinant of adipogenic commitment. Depending on the activation of either canonical or non-canonical WNT pathways, MSCs may either retain their precursor identity or commit to adipogenesis. The canonical WNT pathway maintains the precursor state and inhibits adipogenesis, while the non-canonical pathway, in contrast, supports adipogenic differentiation [67, 72–74]. Interestingly, there is a non-canonical WNT pathway that mimics the canonical pathway by inhibiting adipogenesis, and this dichotomy is further fine-tuned by ligand types. For example, WNT5A activates the anti-adipogenic non-canonical pathway, whereas WNT5B promotes the pro-adipogenic non-canonical pathway [74, 75]. Furthermore, the relative abundance of these ligands influences the outcome [76], highlighting the intricate regulation of WNT signaling in MSC commitment.

In addition to WNT signaling, the TGF- β superfamily of molecules, including TGF- β and bone morphogenetic protein 4 (BMP4), significantly impacts MSC commitment to adipogenesis [77]. While TGF- β and BMP4 have overlapping signaling features, their effect on adipogenesis is divergent: TGF- β signaling is

inhibitory for adipogenesis, whereas BMP4 promotes it [78, 79]. Upon binding to its receptor, TGF- β activates Smad2/3 proteins, which inhibit the transcription of key adipogenic transcription factors [78, 80, 81]. Conversely, BMP4 signaling activates Smad4, which stimulates the transcription of peroxisome proliferator-activated receptor γ (PPAR γ), a critical regulator of adipogenesis, via the zinc finger protein 423 (ZNF423) [82]. The contrasting effects of TGF- β and BMP4 on adipogenesis align with observed patterns of adiposity. In obesity, there is an impairment in the ability to recruit and differentiate progenitor cells, thus exacerbating the pathological adipose tissue environment (Figure 1.4) [59]. Interestingly, BMP4 levels are elevated in adipose tissue from individuals with obesity, suggesting its role in compensating for impaired adipogenesis. However, increased levels of BMP4 antagonists in obesity lead to BMP4 resistance [83]. Similarly, TGF- β levels rise in obesity and may contribute to the excessive storage of lipids in already mature adipocytes by inhibiting the differentiation of new adipocytes [84]. Indeed, strategies to block TGF- β show protection from obesity [85].

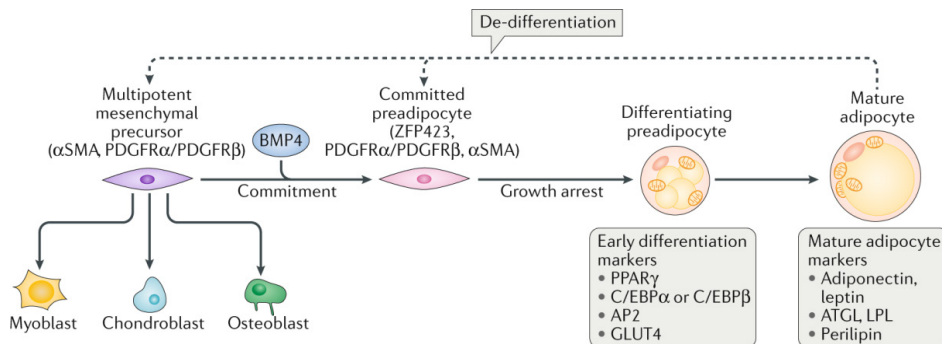


Figure 1.5. Molecular mechanisms of adipogenesis

Adipogenesis is the process through which multipotent mesenchymal precursors (also known as mesenchymal stem cells, MSCs) differentiate into mature adipocytes. It begins with the commitment of MSCs to the adipocyte lineage, resulting in committed preadipocytes. As these preadipocytes differentiate, they activate key transcription factors, including PPAR γ and C/EBP α/β , which drive lipid accumulation and the development of the adipocyte phenotype. Reprinted with permission [66].

Once MSCs have committed to the adipocyte lineage, they progress through key stages that further drive their differentiation into mature adipocytes. These stages involve critical events such as mitotic clonal expansion (MCE), where preadipocytes undergo synchronized cell division, setting the stage for the subsequent acquisition of mature adipocyte characteristics. The regulation of

MCE is crucial for ensuring proper adipogenesis and serves as a pivotal step in the transition to terminal differentiation.

1.2.4.2 Mitotic Clonal Expansion

MCE represents a critical early step in adipogenesis, occurring after growth-arrested preadipocytes are stimulated to differentiate. During this process, preadipocytes synchronously re-enter the cell cycle and undergo several rounds of cell division before permanently exiting the cycle [69, 79, 86, 87]. This transient period of proliferation is necessary for differentiation to proceed: blocking MCE results in impaired or incomplete adipogenesis [69, 88]. While the exact mechanisms linking MCE to differentiation are not fully understood, it is thought that MCE primes preadipocytes for adipogenesis by altering chromatin accessibility at promoter and enhancer regions, thereby enabling the binding of key adipogenic transcription factors such as PPAR γ and C/EBPs [88].

Interestingly, MCE also appears to play a role in balancing the pool of preadipocytes within adipose tissue. During the G1 phase of the cell cycle, a competitive interaction between cyclin D1 and p21 determines whether preadipocytes will differentiate or continue to proliferate [87]. This balance may be particularly relevant in conditions of metabolic dysfunction, where dysregulated adipogenesis contributes to pathological changes in adipose tissue.

In obesity, the capacity for adipose tissue expansion through the recruitment and differentiation of preadipocytes is impaired, contributing to ectopic fat deposition and systemic metabolic disturbances (Figure 1.4) [59]. While the role of MCE in this context is not fully established, it is possible that disruptions in MCE could limit the generation of new, metabolically healthy adipocytes, exacerbating insulin resistance and systemic metabolic dysfunction.

Ultimately, by facilitating both the expansion of committed cells and the priming of transcriptional regulators, MCE serves as a critical preparatory phase for the subsequent transition to terminal differentiation.

1.2.4.3 Terminal Differentiation

Terminal differentiation marks the final stage of adipogenesis, where a committed MSC, now a preadipocyte, will complete the transition to a mature adipocyte. This process is regulated by a variety of transcription factors and signaling pathways, with the expression C/EBPs and PPAR γ being the key drivers

of terminal differentiation. These transcription factors initiate the expression of genes necessary for the acquisition of the adipocyte phenotype, including those involved in lipid storage, metabolic regulation, and insulin sensitivity.

1.2.4.4 CCAAT/enhancer-binding proteins (C/EBPs)

C/EBPs are a family of transcription factors that play critical roles in regulating gene expression, particularly in adipose tissue and the liver. Among the family, C/EBP α , β , and δ are particularly important in adipogenesis. These transcription factors are expressed in a sequential and overlapping manner during adipocyte differentiation, with C/EBP β and δ being expressed early in terminal differentiation, followed by C/EBP α . Once expressed, C/EBPs bind to CCAAT motifs in the DNA to regulate the expression of adipogenic target genes.

In the early stages of adipogenesis, the levels of C/EBP β and δ are initially low in preadipocytes. However, following pro-adipogenic stimulation, their expression increases significantly. Notably, despite the rapid increase in C/EBP β and δ expression, their DNA binding activity does not occur immediately. There is a lag period of approximately 14 hours after stimulation, during which time the expression of two important targets, C/EBP α and PPAR γ , is delayed [89–92]. This lag period is essential because both C/EBP α and PPAR γ are antimitotic [93–98], meaning that their premature expression would impair MCE and thus inhibit adipogenesis. Once C/EBP β becomes active, it interacts with the promoter regions of the terminal differentiation markers, C/EBP α and PPAR γ [89, 90, 92, 99].

As terminal differentiation progresses, C/EBP β and δ expression decreases and is gradually replaced by C/EBP α [90, 99, 100]. C/EBP α then initiates a feedforward loop, upregulating its own expression and that of PPAR γ , a key transcription factor in terminal differentiation. The activation of both C/EBP α and PPAR γ is essential for the full maturation of adipocytes and the establishment of their metabolic functions.

1.2.4.5 Peroxisome proliferator-activated receptor γ (PPAR γ)

PPAR γ , a member of the nuclear hormone receptor superfamily, is a critical transcription factor required to drive adipogenesis [101, 102]. It is often referred to as the ‘master regulator’ of adipogenesis due to its pivotal role in orchestrating the gene expression program necessary for preadipocytes to fully differentiate into mature adipocytes. As differentiation progresses, PPAR γ

expression reaches a threshold that triggers several positive feedback loops, sustaining its levels even in the absence of external stimuli [87].

One of the key mechanisms by which PPAR γ regulates adipogenesis is by acting as an irreversible switch following MCE. This is achieved through PPAR γ -driven expression of the cyclin-dependent kinase (CDK) inhibitor p21, which helps to solidify the transition from cell division to terminal differentiation [87]. In combination with C/EBP α , PPAR γ activates the expression of genes involved in lipid metabolism, fatty acid uptake, insulin sensitivity, and other critical functions of mature adipocytes. This cooperative action between PPAR γ and C/EBP α ensures the establishment of adipocyte identity and the proper functioning of adipose tissue.

1.2.4.6 Summary of Adipogenesis

Adipogenesis is a highly regulated process essential for proper adipose tissue function and metabolic health. It involves commitment to the adipocyte lineage, MCE, and terminal differentiation, with key transcription factors such as C/EBP α and PPAR γ driving the expression of genes involved in lipid metabolism and insulin sensitivity (Figure 1.5). While these core transcription factors are crucial, adipogenesis is also influenced by additional factors, including Notch signaling, insulin and thyroid hormones, extracellular matrix composition, and epigenetic modifications [60, 61, 63, 66, 103]. Disruptions in any phase can impair adipose tissue function and contribute to metabolic disorders like obesity and insulin resistance.

1.2.5 Neural Regulation of Adipose Tissue

The neural regulation of adipose tissue is a critical component in controlling adipose tissue function and overall energy balance and metabolism [104–107]. Adipose tissue is innervated by both sympathetic and sensory nerve fibers, each with distinct functional roles (Figure 1.6). Research to date has focused predominantly on the sympathetic nervous system (SNS), which regulates processes like lipolysis and thermogenesis. In contrast, sensory innervation, though less well-studied, plays a key role in relaying information from adipose tissue to the central nervous system (CNS) [105]. While there is a consensus that parasympathetic innervation is absent or negligible in adipose tissue [108], understanding the contributions of sympathetic and sensory pathways is essential for elucidating adipose tissue biology and metabolism.

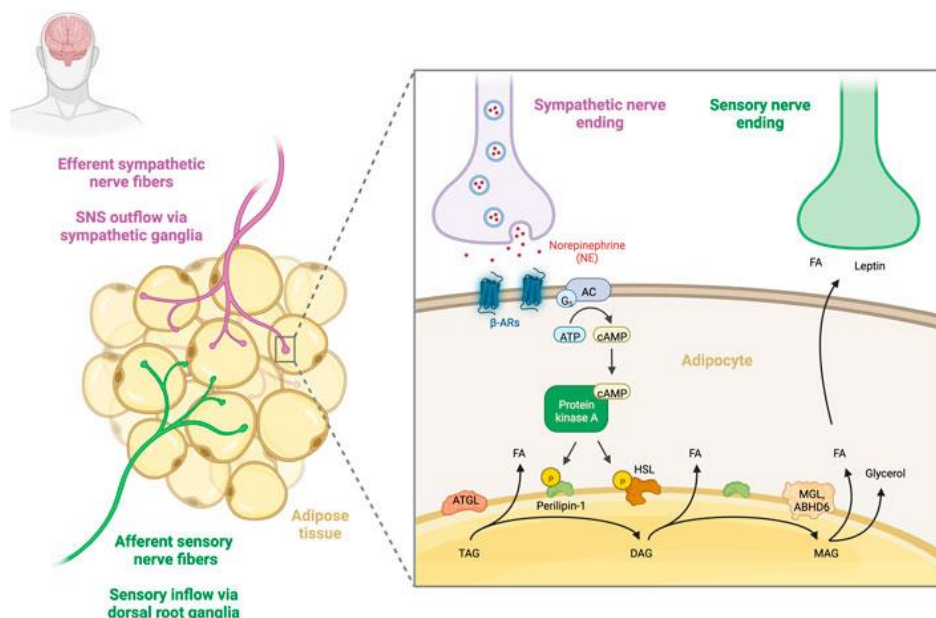


Figure 1.6. Neural innervation of adipose tissue

Sympathetic and sensory innervation of adipose tissue. Sympathetic nerve fibers (purple) transmit signals from the central nervous system (CNS) to adipose tissue, releasing norepinephrine (NE) to activate β -adrenergic receptors. This triggers a cascade involving Gs protein activation, adenylate cyclase (AC), and increased cAMP levels, which activate protein kinase A (PKA). PKA phosphorylates hormone-sensitive lipase (HSL) and perilipin-A (PLIN1), initiating triglyceride (TG) breakdown by lipases such as adipose triglyceride lipase (ATGL) and monoglyceride lipase (MGL). This releases fatty acids (FA) and glycerol. Sensory nerve fibers (green) relay feedback to the CNS, sensing local FA and leptin levels. Reprinted under the Creative Commons Attribution License (CC BY) [109].

1.2.5.1 Sympathetic Innervation of Adipose Tissue

The sympathetic nervous system (SNS) plays a pivotal role in regulating adipose tissue function. While both BAT and WAT are innervated by sympathetic fibers, their physiological roles differ substantially. BAT is primarily associated with thermogenesis, driven by dense sympathetic input and the activation of UCP1 [110]. In contrast, the sympathetic innervation of WAT governs processes such as lipolysis, proliferation, and beiging [111–113]. Given the central role of WAT in metabolic health, this section focuses on the mechanisms through which the SNS influences WAT function.

The recognition of SNS innervation in WAT dates back to 1898 [114], when nerve fibers of unknown origin were first reported within the tissue. Since then, accumulating evidence has established that WAT is appreciably innervated by the SNS [112, 113, 115]. Early studies focused predominantly on the SNS's role in regulating blood vessels within WAT, given its then-established presence

surrounding the vasculature [116]. However, advances in imaging techniques have since revealed direct sympathetic innervation of adipocytes, highlighting a broader role in adipose tissue regulation [117].

One of the primary functions of sympathetic innervation of WAT is the regulation of lipid metabolism through lipolysis—the breakdown of triglycerides into free fatty acids (FFAs) and glycerol [106]. This process begins when catecholamines, particularly norepinephrine (NE), are released from sympathetic nerve terminals. NE binds to β -adrenergic receptors (β -ARs) on the surface of adipocytes, triggering a cascade of intracellular signaling events (Figure 1.6) [118]. This includes the activation of adenylate cyclase and subsequent elevation of cyclic AMP (cAMP) levels, leading to the activation of protein kinase A (PKA) [118]. PKA phosphorylates and activates hormone-sensitive lipase (HSL) and other lipases, facilitating the hydrolysis of stored triglycerides [118]. The FFAs released during this process serve as a critical energy source for peripheral tissues and help regulate systemic metabolism (Figure 1.6).

Beyond its role in lipid metabolism, the SNS is also instrumental in promoting the beiging of white adipocytes—the process by which white adipocytes acquire characteristics of brown adipocytes [110]. This transformation is an evolutionary adaptation to generate heat, allowing mammals to maintain body temperature under cold stress [31]. During beiging, WAT undergoes significant morphological and functional changes, including increased mitochondrial content, enhanced energy expenditure, and upregulation of thermogenic genes such as UCP1. This process is regulated in part by the release of NE from sympathetic terminals, which binds to β -ARs on adipocytes and activates signaling pathways that drive thermogenic gene expression [118]. Key players in this pathway include peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) and fibroblast growth factor 21 (FGF21), which promote mitochondrial biogenesis and thermogenic programming [119]. Given its potential to increase energy expenditure and improve metabolic health, beiging has emerged as a promising strategy to address obesity-related disorders [120].

A lesser recognized role of sympathetic innervation of WAT is the regulation of preadipocyte proliferation. Early research demonstrated that NE inhibits preadipocyte proliferation in vitro, an effect blocked by β -AR antagonists [121]. In vivo, sympathetic denervation of WAT increases fat cell number [112, 113, 122, 123], further supporting the role of the SNS in controlling preadipocyte proliferation.

However, recent findings have added complexity to this understanding. Neuropeptide Y, a neuropeptide secreted by sympathetic neurons, sustains the proliferation of a subset of mural cells—a vascular cell type that contributes to the formation of thermogenic adipocytes [124]. Together, these findings highlight the multifaceted role of the SNS in WAT, regulating not only lipid mobilization and energy expenditure but also adipocyte proliferation and tissue remodeling.

1.2.5.2 Sensory Innervation of Adipose Tissue

The sensory innervation of adipose tissue was first anatomically documented in 1985, when substance P (SP), a sensory nerve-associated peptide, was identified in the nerves supplying adipose tissue [125]. This was further corroborated by anterograde tracer studies, which labeled pseudo-unipolar neurons in the dorsal root ganglia—the anatomical location of sensory neuron cell bodies [126].

Subsequently, another sensory peptide, alpha-calcitonin gene-related peptide (CGRP α), was detected in adipose tissue across multiple species [127, 128].

Advances in imaging technologies, such as whole mount confocal microscopy [117, 129] and tissue clearing techniques [130], have further solidified the presence of sensory innervation in adipose tissue, providing unequivocal evidence that sensory nerves play an integral role in adipose tissue biology.

While the sensory innervation of adipose tissue has been well-documented anatomically, its functional significance is still not fully understood. Early experiments suggested that sensory nerves might mediate a reflex arc between adipose tissue and the CNS (Figure 1.7) [131, 132]. For instance, leptin—a hormone secreted by adipocytes and proportional to body fat [133]—injections into adipose tissue increased the electrophysiological activity of afferent nerves (Figure 1.6) [132], which was shown to induce lipolysis in contralateral depots via sympathetic drive [131]. Subsequent studies have expanded this work, showing that the sympathetic response to sensory activation may not be specific to adipose tissue but could represent a broader systemic activation (Figure 1.7) [134, 135]. Additionally, both SNS-induced lipolysis and fatty acid injections into adipose tissue have been found to stimulate sensory afferents, pointing to the existence of endogenous stimulants that activate adipose tissue afferents (Figure 1.6) [136, 137]. This underscores the possibility that additional, yet-to-be-discovered stimulants could play a role in regulating energy balance by communicating body fat levels to the brain, opening new therapeutic avenues for improving whole-body metabolism.

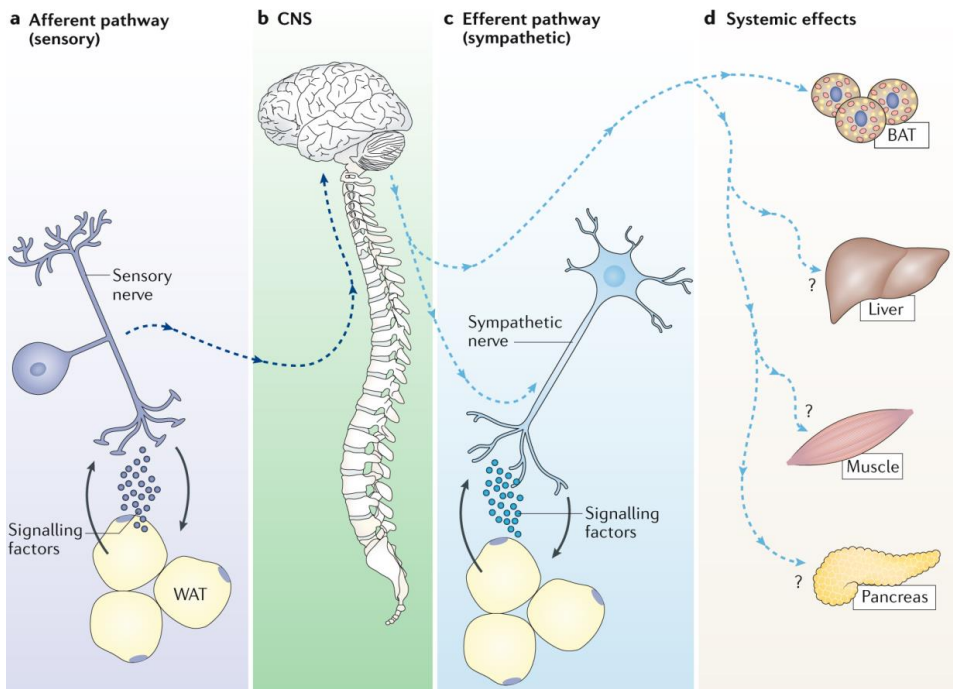


Figure 1.7. Sensory innervation of adipose tissue and crosstalk with the CNS

(A) Sensory nerves detect signals in WAT and release local factors that modulate the microenvironment. At the same time, these sensory neurons relay information from the WAT to the CNS. (B) The CNS integrates these signals to generate a coordinated response. (C) Sympathetic nerves convey this response back to the adipose tissue, releasing signaling molecules such as norepinephrine, which modulate the local microenvironment. (D) The autonomic nervous system also affects other metabolic organs, such as the liver, muscle, and pancreas, to promote systemic homeostasis. Whether adipose-derived signals directly influence CNS-driven sympathetic regulation of these organs (light blue arrows) remains unclear. This schematic illustrates how the adipose tissue microenvironment might regulate systemic metabolism through signaling to local nerve fibers. Reprinted with permission [105].

Studies exploring sensory nerve function have utilized various models, including chemical and surgical denervation, lipectomy, and genetic manipulation.

Chemical denervation, which involves the administration of neurotoxic compounds like capsaicin to destroy sensory afferents, results in an increase in the size of sensory-intact adipose depots [128], which suggests that sensory afferents contribute to regulating adiposity. Increased leptin, a signal of higher adiposity, typically drives SNS-mediated lipolysis to reduce fat stores. However, when sensory input is lost, this signal appears to be absent, leading to a reduction in sympathetic drive to adipose tissue. This shift away from lipolysis supports the idea that the loss of sensory input signals reduced adiposity and triggers energy conservation, ultimately promoting fat accumulation [138]. However, conflicting results regarding fat pad mass changes following sensory

denervation suggest the need for cautious interpretation of these findings [122, 130, 139].

Compared to chemical denervation, surgical denervation involves lacerating nerve bundles containing both sensory and sympathetic nerves [128]. After surgical denervation, adipose depots increase in size by up to 50% [122, 128]. The increased size is reflected by an increase in cell number, which is thought to result from the loss of sympathetic inhibition of adipocyte proliferation [112, 121, 140]. Interestingly, while sensory denervation alone does not appear to stimulate adipocyte proliferation, sympathetic denervation does [113].

Lipectomy provides an indirect method to study sensory afferents by removal of fat mass while preserving innervation. Lipectomy of epididymal adipose tissue triggers compensatory increases in other fat depots [128, 138], accompanied by a significant reduction in NE turnover in brown adipose tissue—indicating a shift to an energy-conserving state. These observations are consistent with the idea that sensory denervation triggers a similar process of fat pad expansion to restore body fat stores.

More recent studies have shown that selective deletion of TRPV1-expressing sensory neurons not only promotes a youthful metabolic profile and extends lifespan—by reducing CGRP α release from pancreatic islet-innervating fibers and thereby enhancing insulin secretion—but also confers systemic metabolic benefits [141]. Complementing these findings, targeted ablation of heat-sensing CGRP α neurons increases energy expenditure, enhances lipid oxidation, and protects against high-fat diet-induced weight gain by promoting adaptive thermogenesis [142]. Furthermore, selective genetic ablation of sensory nerves innervating iWAT results in increased de novo lipogenesis, fatty acid metabolism, and cold-induced thermogenesis, with these effects being partially dependent on intact sympathetic innervation [130, 142]. Sensory neurons expressing the mechanosensor Piezo2 were recently shown to regulate thermogenic programs in both brown and beige fat, with their effects mediated by the inhibition of NE release from sympathetic neurons [143]. Collectively, these findings suggest that sensory neurons play a broader role in whole-body energy metabolism while specifically regulating sympathetic activity in adipose tissue.

1.2.5.3 *Efferent Properties of Sensory Efferents*

While sensory afferents transmit information to the central nervous system, they also possess efferent properties. When activated, sensory neurons release

neuropeptides locally (Figure 1.7) [144, 145]. Two neuropeptides of particular interest are substance P (SP) and alpha-calcitonin gene-related peptide (CGRP α), both of which are well-known for their roles in cardiovascular physiology, though their effects on adipose tissue remain less understood.

CGRP α is widely recognized for its vasodilatory function and its involvement in migraine pathophysiology [146]. Interestingly, CGRP α levels are elevated in various models of obesity [147–150], a condition characterized by increased circulating leptin [133, 151] and free fatty acids [152]—both of which stimulate sensory neurons. In the adipose tissue microenvironment where the sensory neuron is located, CGRP α concentrations may therefore be higher than what is seen in circulation. This raises the possibility that the elevated circulating CGRP α seen in models of obesity may be partly due to increased secretion of CGRP α from sensory neurons within adipose tissue. However, the local effects of CGRP α in adipose tissue remain largely unexplored. One study demonstrated that CGRP α promoted lipolysis in 3T3–L1 adipocytes [153]. Others have shown that by reducing circulating CGRP α levels, energy expenditure increases, and improved weight loss is observed [141, 142, 147, 154, 155]. These studies, however, did not address the localized effects of sensory neuron-derived CGRP α in adipose tissue, which may have distinct roles compared to circulating levels.

Similarly, SP, another neuropeptide associated with vasodilation [156], has also been linked to obesity [157] and is involved in a variety of metabolic processes. SP has been shown to promote a pro-inflammatory environment [158, 159], support preadipocyte proliferation and anti-apoptotic pathways [160], impair insulin-stimulated glucose uptake in mature adipocytes [161], and reduce lipid accumulation during adipogenesis [159], though the effects are not always consistent [162]. Interestingly, models that reduce SP signaling have been shown to reduce weight gain in high-fat diet-fed mice and improve glucose clearance [161, 163]. These findings suggest that SP may contribute to the pathogenesis of metabolic disease, yet the specific role of sensory neuron-derived SP within adipose tissue remains unclear.

While the individual effects of SP and CGRP α on metabolism have been partially explored, sensory neurons release a variety of other neuropeptides [164–168], which likely have complementary or even opposing roles in regulating adipose tissue. The relative proportions of these neuropeptides released in response to different stimuli add another layer of complexity to the regulation of adipose

tissue by sensory afferents [144, 169, 170]. Although these complexities are likely crucial for the communication between sensory neurons and adipose tissue, much remains unknown. The field is still in the early stages of understanding how these neuropeptides interact and the extent of their physiological relevance in adipose tissue biology [156, 164, 171].

1.3 Skeletal Muscle

Skeletal muscle is a dynamic and multifunctional tissue, essential not only for locomotion and physical movement but also for maintaining metabolic homeostasis and overall health. Comprising nearly 40% of total body mass in adults, skeletal muscle serves as a primary site for glucose uptake and storage, energy expenditure, and protein synthesis. Its ability to adapt to a wide range of physiological and environmental demands—such as exercise, injury, and disease—highlights its remarkable plasticity.

In this section, we will explore the fundamental biology of skeletal muscle, beginning with its role in health, including its structural organization, adaptability, and contributions to metabolic regulation. We will then examine how skeletal muscle dysfunction contributes to disease states such as sarcopenia, muscular dystrophy, and cachexia, with a focus on the molecular pathways driving muscle atrophy. Next, we will discuss the processes underlying muscle repair and regeneration, emphasizing the interplay between satellite cells, immune responses, and extracellular matrix remodeling. Finally, we will consider therapeutic strategies aimed at restoring muscle function in conditions characterized by muscle dysfunction. Understanding the intricate biology of skeletal muscle provides insight into its vital roles in health and disease and reveals opportunities for therapeutic intervention.

1.3.1 Skeletal Muscle in Health

Skeletal muscle is a highly organized and adaptable tissue that plays a central role in movement, metabolism, and overall health. Structurally, it is composed of muscle fibers grouped into fascicles, surrounded by connective tissue layers that provide support and facilitate force transmission [172]. Each fiber is multinucleated, containing myofibrils that enable contraction through the interaction of actin and myosin filaments [172]. This precise organization enables skeletal muscle to generate force and maintain postural stability, vital for locomotion and daily activities.

Muscle fibers are categorized based on their contractile and metabolic properties, spanning a spectrum from glycolytic fast-twitch fibers (type II) to oxidative slow-twitch fibers (type I) [173]. Type II fibers, which rely on anaerobic glycolysis, provide rapid and powerful contractions but are prone to fatigue. In contrast, type I fibers rely on oxidative phosphorylation, supporting sustained contractions with greater fatigue resistance [173].

Beyond its structural and mechanical functions, skeletal muscle is a critical regulator of metabolic homeostasis [174]. It serves as the body's largest reservoir for glucose disposal during insulin-stimulated conditions [175]. This capacity to absorb and store glucose plays a pivotal role in maintaining blood glucose homeostasis and energy balance [176].

Skeletal muscle is also highly plastic, responding dynamically to various physiological demands. Mechanical overload, such as resistance training, induces hypertrophy through the activation of signaling pathways like mTORC1, leading to increased protein synthesis and muscle fiber growth [177]. In contrast, aerobic exercise enhances mitochondrial biogenesis and oxidative capacity, largely mediated by PGC-1 α activation [178]. Conversely, muscle disuse during immobilization or bed rest triggers rapid atrophy, driven by the activation of catabolic pathways like the ubiquitin-proteasome system and autophagy [172].

In addition to its intrinsic properties, skeletal muscle communicates with other tissues via the secretion of myokines, bioactive molecules that influence systemic processes such as inflammation and energy metabolism. For example, interleukin-6 (IL-6), released during exercise, enhances glucose uptake and lipolysis in adipose tissue while promoting anti-inflammatory effects [15, 174, 179]. As another example, brain-derived neurotrophic factor (BDNF), released by skeletal muscle during exercise, supports neurogenesis and enhances lipid oxidation in skeletal muscle [180]. These examples highlight the systemic impact of skeletal muscle beyond its intrinsic properties.

While skeletal muscle's adaptability is critical for its function, this same plasticity renders it susceptible to degeneration during periods of disuse or disease. The next section examines how skeletal muscle atrophy contributes to pathological states, exploring the molecular pathways and experimental models that provide insight into these processes.

1.3.2 When Muscle Misfires: Skeletal Muscle in Disease

Skeletal muscle dysfunction is a hallmark of numerous pathological conditions, manifesting as the progressive loss of muscle mass and function—a condition termed muscle atrophy. Atrophy is a common feature of aging (sarcopenia), chronic diseases (e.g., cancer cachexia, chronic obstructive pulmonary disease), and periods of mechanical unloading such as bedrest, immobilization, or microgravity. This loss of muscle mass and function not only impairs mobility and quality of life but also disrupts systemic metabolism, exacerbating overall

health decline. Muscle atrophy is a multifactorial process influenced by a range of molecular systems, including oxidative stress, inflammation, and mitochondrial dysfunction [181]. These factors initiate signaling pathways that converge on mechanisms driving protein degradation, ultimately contributing to muscle wasting.

Central to the muscle atrophy process, the ubiquitin-proteasome system (UPS) regulates the selective degradation of myofibrillar proteins [181]. This system is triggered by various catabolic signals that enhance the activity of muscle-specific E3 ubiquitin ligases, such as atrogin-1 (MAFbx) and muscle ring finger-1 (MuRF1) [181]. These ligases are transcriptionally upregulated by Forkhead box O (FOXO) transcription factors, which are activated in response to stress signals like glucocorticoids and pro-inflammatory cytokines, including TNF- α and IL-6 [182]. Elevated glucocorticoid levels, often seen during chronic disease states, along with cytokine-induced inhibition of Akt signaling [183], further activate FOXO transcription factors. This suppression of Akt signaling not only promotes FOXO activation but also inhibits mTORC1, reducing protein synthesis and exacerbating muscle loss [183]. These pathways converge to upregulate MAFbx and MuRF1, leading to enhanced protein ubiquitination and degradation, ultimately contributing to muscle atrophy and an overall loss of function [181].

In addition to the UPS, autophagy is another critical pathway regulating muscle degradation. While basal autophagy maintains cellular homeostasis by removing damaged organelles and misfolded proteins, excessive autophagic activity exacerbates muscle loss during atrophy. Forkhead box O3 (FoxO3), a key transcriptional regulator, controls several autophagy-related genes, including LC3 and Bnip3 [181, 184]. LC3 is involved in autophagosome formation, facilitating the sequestration of damaged proteins and organelles for degradation, while Bnip3 plays a role in mitophagy, the selective autophagic removal of damaged mitochondria [184]. Dysregulation of these processes, often triggered by catabolic signals like AMPK activation or prolonged glucocorticoid exposure, disrupts muscle homeostasis and accelerates the degradation of cellular components, contributing to the progression of muscle atrophy.

Beyond the UPS and autophagy, proteolytic enzymes like calpains and caspases contribute significantly to muscle atrophy [181]. Calpains are calcium-dependent proteases that cleave various substrates, including structural proteins like desmin and α -actinin, thereby compromising muscle integrity [181]. Caspases,

particularly caspase-3, also contribute to muscle degradation by cleaving myofibrillar proteins such as actomyosin [181]. Their actions, together with those of the UPS and autophagy, create a proteolytic cascade that amplifies the degradation of muscle proteins, further accelerating muscle loss.

Lastly, underlying chronic inflammation plays a pivotal role in muscle atrophy, acting synergistically with the previously discussed mechanisms to exacerbate muscle degradation. Among the cytokines involved, IL-6 and TNF- α play prominent roles. IL-6 activates the STAT3 pathway, which modulates several signaling cascades, including JAK/STAT3, ERK, and PI3K/Akt, ultimately promoting muscle wasting [185]. TNF- α , on the other hand, binds to its receptor and activates the IKK complex, leading to the phosphorylation, ubiquitination, and degradation of I κ B proteins [185]. This process frees NF- κ B to translocate to the nucleus, where it initiates the expression of genes involved in muscle atrophy.

The interplay of inflammation, proteolytic enzyme activation, and impaired protein turnover creates a cascade that accelerates muscle atrophy. This network of signaling pathways, involving the UPS, autophagy, calpains, and caspases, drives the degradation of muscle proteins and further compromises muscle function. Chronic inflammation amplifies these processes, creating a vicious cycle that not only leads to muscle wasting but also worsens the progression of disease, contributing to diminished mobility, reduced quality of life, and increased vulnerability to further complications. Despite significant advances, this complex process remains incompletely understood, requiring further exploration to fully elucidate its mechanisms and implications.

1.3.3 Muscle Regeneration: From Damage to Repair

Muscle regeneration is a critical biological process that restores muscle tissue following injury or disease. It is a highly dynamic and complex response, encompassing inflammation, immune cell recruitment, satellite cell activation, and tissue remodeling. In this process, three key stages contribute to successful tissue repair: injury and inflammation, proliferation and differentiation of satellite cells, and remodeling and maturation of the regenerating muscle tissue. Here, we will explore these stages, focusing on the molecular mechanisms that drive each phase.

1.3.3.1 Injury and Inflammation

The initiation of muscle regeneration begins with injury, which can manifest acutely, such as through physical trauma, exercise-induced microtears, or chemical damage, or chronically, as in degenerative diseases like muscular dystrophy (Figure 1.8). Tissue injury triggers the release of intracellular signals known as damage-associated molecular patterns (DAMPs), which play a critical role in activating the immune response [186].

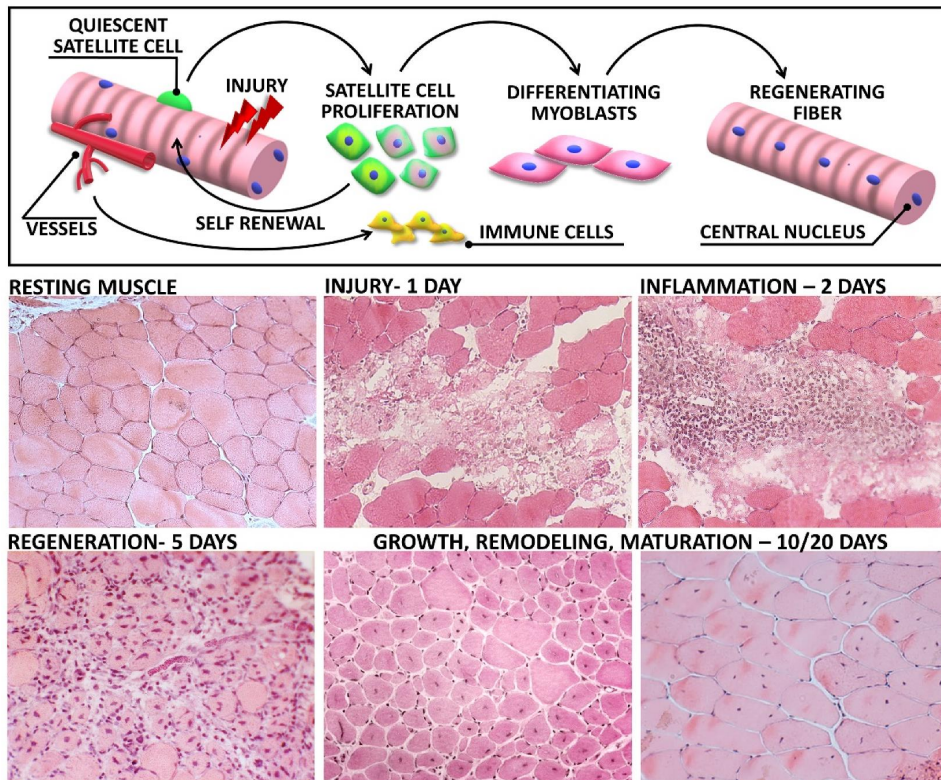


Figure 1.8. Skeletal muscle regeneration after acute injury

The top panel illustrates key biological responses following muscle damage, while the bottom panel shows histological images of muscle regeneration after acute muscle damage. One day post-injury, necrotic fibers are visible, followed by an inflammatory response on day two. Activated satellite cells proliferate, leading to the formation of new muscle fibers within the first week. By day ten, muscle structure is largely restored, with centrally located nuclei marking regeneration. Over time, fibers continue to grow and mature, with nuclei shifting toward the periphery. Reprinted under the Creative Commons Attribution License (CC BY) [186].

The inflammatory response, though often associated with negative connotations, is an essential component of muscle repair. Among the first responders to the site of damage are neutrophils and mast cells [186]. Mast cells release pro-

inflammatory cytokines, including TNF- α , interleukin-1 beta (IL-1 β), and interferon-gamma (IFN- γ), which together stimulate the recruitment of peripheral neutrophils to the site of injury [187]. Neutrophils are pivotal in removing necrotic debris and amplifying the inflammatory response by releasing additional pro-inflammatory mediators that stimulate subsequent waves of immune cell infiltration [187].

As the inflammatory process progresses, macrophages gradually replace neutrophils in the damaged tissue [187]. Initially, phagocytic macrophages (historically referred to as M1 macrophages) work to clear cellular debris and sustain the pro-inflammatory environment. Over time, a shift occurs towards non-phagocytic macrophages (sometimes described as M2 macrophages), which are thought to promote the resolution of inflammation and initiate tissue repair through the secretion of anti-inflammatory cytokines and growth factors [187]. Although the strict M1/M2 dichotomy remains controversial, it is widely accepted that macrophages play a dual role in muscle regeneration, first as initiators of inflammation and later as mediators of repair [186].

These interrelated and time-dependent waves of immune cell activity are essential for transitioning from inflammation to tissue regeneration, setting the stage for the activation of satellite cells and subsequent phases of repair and remodeling.

1.3.3.2 Proliferation and Differentiation of Satellite Cells

Following the inflammatory response, satellite cells—muscle-specific stem cells—play a central role in the regeneration process. Satellite cells reside in a quiescent state beneath the basal lamina of muscle fibers, characterized by the expression of markers such as Pax7 [186]. Upon injury, they are activated by signaling molecules released from the damaged tissue and infiltrating immune cells, including hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and insulin-like growth factor-1 (IGF-1) [188]. These signals trigger the transition of satellite cells from quiescence to an activated state, initiating their proliferation to expand the myogenic progenitor pool (Figure 1.8).

During the proliferative phase, satellite cells downregulate Pax7 and upregulate myogenic regulatory factors (MRFs) such as MyoD and Myf5, which commit them to the myogenic lineage [186]. This phase is tightly regulated by both intrinsic factors, such as cell-cycle regulators, and extrinsic cues, including Wnt and Notch signaling [188, 189]. Notch signaling, in particular, maintains the

undifferentiated state of proliferating satellite cells, delaying premature differentiation and ensuring an adequate pool of progenitors for regeneration [189].

As satellite cells proliferate, a subset begins to exit the cell cycle and initiates differentiation, marked by the expression of myogenin and MyoD [186].

Differentiated myogenic progenitors, or myoblasts, begin to fuse with one another and with existing myofibers, restoring the structural and functional integrity of the muscle. The fusion process is mediated by a cascade of events involving actin cytoskeleton remodeling and the expression of fusogenic proteins such as myomaker and myomerger [190].

Importantly, while most satellite cells contribute to the repair process, a subset returns to quiescence to replenish the satellite cell pool, ensuring the muscle's regenerative capacity for future injuries. This balance between proliferation, differentiation, and self-renewal is critical and relies on finely tuned interactions between signaling pathways, such as transforming growth factor-beta (TGF- β) and bone morphogenetic proteins (BMPs) [186, 187]. Dysregulation of these processes can lead to impaired regeneration, as observed in chronic muscle diseases and aging.

The interplay of these molecular and cellular events underscores the importance of satellite cells in orchestrating the repair and renewal of damaged muscle tissue, bridging the inflammatory phase with the final stages of tissue remodeling.

1.3.3.3 *Remodeling and Maturation*

The final stage of muscle regeneration involves the remodeling and maturation of tissue to restore both structural integrity and functional capacity. During this phase, newly formed myofibers increase in size, align with the existing muscle architecture, and replace embryonic myosin heavy chain (eMyHC) with adult isoforms, acquiring the contractile properties characteristic of mature muscle fibers. The maturation process is further evidenced by the peripheral placement of nuclei within myofibers, a hallmark of fully developed muscle tissue (Figure 1.8) [186].

Beyond myofiber maturation, the restoration of functional performance relies on the reestablishment of critical support systems. Angiogenesis, driven by vascular endothelial growth factor (VEGF), ensures an adequate blood supply, while

reinnervation restores communication between muscle fibers and the nervous system [191]. The successful integration of regenerated myofibers with neuromuscular junctions (NMJs) is essential for enabling proper signal transmission and contractile performance, bridging the gap between structural repair and functional recovery [186].

The extracellular matrix (ECM) undergoes substantial remodeling to provide structural support and facilitate the integration of newly formed fibers with pre-existing ones. Fibroblasts play a central role in this process, depositing collagen and other matrix proteins under the influence of regulatory factors such as TGF- β . While controlled ECM deposition forms a beneficial scaffold, excessive fibrosis can result in scarring and impaired muscle function [186].

Ultimately, the coordinated maturation of myofibers, ECM remodeling, angiogenesis, and reinnervation ensures the restoration of muscle strength and resilience, marking the completion of the regenerative cycle.

1.3.4 Therapeutic Implications: Restoring Muscle Function

In conditions such as sarcopenia, cachexia, and muscular dystrophies, the regenerative processes in muscle tissue are often disrupted. Sarcopenia, for example, is characterized by diminished satellite cell function and a reduced ability to repair muscle damage [172]. Cachexia, on the other hand, is driven by systemic inflammation, which exacerbates muscle wasting and impairs the regenerative response [192]. In muscular dystrophies, genetic defects in muscle fibers or satellite cells prevent normal muscle regeneration, leading to progressive loss of muscle function [186]. These diseases share a common feature: an impaired ability to activate satellite cells, control inflammation, and remodel the ECM, all of which are essential for efficient muscle repair and regeneration.

One approach to enhancing muscle regeneration is modulating inflammation, which is particularly relevant in conditions like cachexia, where chronic inflammation accelerates muscle loss [185–187]. Inhibiting pro-inflammatory cytokines like TNF- α can reduce muscle wasting and promote muscle repair. Targeting inflammatory signaling pathways, such as NF- κ B, could also create a more favorable environment for muscle regeneration, as NF- κ B regulates various inflammatory mediators involved in muscle degradation.

In conditions like sarcopenia, where satellite cell activation is impaired, stimulating these cells is crucial for muscle repair. Myostatin inhibitors, such as follistatin, promote muscle growth by blocking the negative regulation of satellite cells, helping to overcome the limitations of satellite cell function in aged or diseased muscle [185]. Another promising strategy involves the use of exercise mimetics like irisin, a myokine that promotes satellite cell activation and muscle regeneration without requiring physical exercise [181, 193].

Muscular dystrophies are marked by excessive fibrosis and impaired ECM remodeling, which impede the regeneration of muscle tissue. Targeting ECM remodeling pathways can help mitigate these issues and restore muscle function [194]. Matrix metalloproteinases (MMPs), which degrade ECM components, have been explored to prevent excessive fibrosis and support muscle regeneration [194]. On the other hand, inhibiting excessive ECM deposition is also important, as overproduction of collagen can lead to scarring and impaired muscle function. At the same time, promoting healthy ECM deposition with specific proteins, such as collagen type IV, can improve the structural integration of newly formed myofibers.

In addition to inflammation and ECM remodeling, gene therapies are being investigated for their potential to restore muscle function in diseases like Duchenne muscular dystrophy (DMD) [195]. For DMD, delivering a functional copy of the dystrophin gene via AAV vectors has shown promise in clinical trials, offering hope for restoring muscle function in affected individuals [195]. Another novel approach involves exosome-based therapies, which can deliver regenerative factors directly to muscle tissue, stimulating satellite cell activation and ECM remodeling in a more controlled manner [196].

In conclusion, therapeutic strategies targeting inflammation, satellite cell activation, and ECM remodeling hold great promise for enhancing muscle regeneration, particularly in diseases where these processes are impaired. However, despite advances in our understanding of the molecular mechanisms driving muscle injury and repair, treatments for conditions such as sarcopenia, cachexia, and muscular dystrophies remain limited and often insufficient. These diseases are characterized by disrupted regenerative processes, and current therapies largely fail to fully restore muscle function. Therefore, there is a pressing need for continued research to identify novel mechanisms and therapeutic targets that could lead to more effective treatments. Ultimately, this

research could help develop therapies that not only improve muscle regeneration but also offer tangible benefits to patients suffering from these debilitating conditions.

1.4 The Kynurenine Pathway of Tryptophan Degradation

The kynurenine pathway is the central route for tryptophan metabolism, accounting for the catabolism of over 90% of dietary tryptophan not used for protein synthesis [197]. While historically recognized for its role in nicotinamide adenine dinucleotide (NAD⁺) biosynthesis, the kynurenine pathway is increasingly understood for its widespread influence on human health [198]. Its bioactive metabolites regulate immune responses, brain function, and energy metabolism, linking this pathway to conditions such as , chronic inflammation, and obesity [197, 198]. In the following sections, we explore the biochemical framework of the kynurenine pathway, its major metabolites, and their biological roles, with a focus on kynurenine, kynurenic acid, and quinolinic acid, particularly in relation to bioenergetics.

1.4.1 Introduction to the Kynurenine Pathway

Tryptophan is an essential amino acid with several metabolic fates. It can be incorporated into proteins, converted into melatonin via the serotonin pathway, or metabolized through the kynurenine pathway. Under normal physiological conditions, most tryptophan is routed through the kynurenine pathway, where a series of enzymatic reactions produces various bioactive metabolites (Figure 1.9) [197]. Notably, the kynurenine pathway is responsible for the de novo synthesis of NAD⁺, emphasizing its central role in maintaining cellular energy balance [197].

The kynurenine pathway begins with the enzymatic degradation of tryptophan by either indoleamine 2,3-dioxygenase (IDO1/IDO2) or tryptophan 2,3-dioxygenase (TDO), depending on the tissue context [197, 198]. This first step converts tryptophan into N-formylkynurenine, which is then rapidly hydrolyzed to kynurenine, the central intermediate of the pathway (Figure 1.9). From kynurenine, the metabolism can take several distinct routes. One route produces kynurenic acid (KYNA) via kynurenine aminotransferases (KATs) [199]. Alternatively, kynurenine can be converted into 3-hydroxykynurenine (3-HK) by kynurenine-3-monooxygenase (KMO), eventually leading to the formation of quinolinic acid (QUIN) [200]. A parallel branch converts kynurenine to anthranilic acid (AA), which also contributes to QUIN production (Figure 1.9). Additionally, KYNA can be generated via an immune-related pathway involving interleukin-4-induced-1 (IL4I1) [201]. Ultimately, the kynurenine pathway results in the production of either KYNA or QUIN, with QUIN serving as a precursor for NAD⁺ (Figure 1.9).

Importantly, only tryptophan, kynurenine, and 3-HK can cross the blood–brain barrier, while downstream metabolites such as KYNA and QUIN remain confined to the periphery. This distinction limits the interpretation of peripheral kynurenine pathway metabolite levels with respect to their functional properties, and it calls for caution when linking systemic kynurenine pathway activity to CNS effects [197].

The kynurenine pathway is not only a central metabolic route but also a dynamic system that can be modulated by various physiological factors. For instance, the kynurenine pathway is particularly responsive to inflammatory signals, with the rate-limiting enzymes IDO1 and KMO being sensitive to proinflammatory cytokines [202, 203]. During acute inflammation, these enzymes are upregulated, driving a compensatory increase in kynurenine metabolism. This helps modulate immune responses and aid in resolving the inflammatory state by producing anti-inflammatory metabolites like KYNA. However, in chronic inflammation, the pathway can shift from compensatory to pathological [197]. Prolonged activation of IDO1 and KMO can lead to the overproduction of neurotoxic intermediates like QUIN and 3-HK, contributing to neuroinflammation and immune dysfunction. Thus, the response of the kynurenine pathway to inflammation is context-dependent, with its role determined by the balance between acute, compensatory activation and chronic, pathological activation.

Beyond inflammation, the kynurenine pathway's role is also influenced by metabolic stressors, including exercise and obesity. Exercise activates the kynurenine pathway in a manner that favors anti-inflammatory and neuroprotective metabolites, such as KYNA [199, 204, 205]. This shift supports the body's adaptation to physical stress by enhancing immune function and promoting neuroprotection, highlighting the pathway's role in maintaining homeostasis during acute stress. On the other hand, obesity, often characterized by low-grade chronic inflammation, leads to dysregulated kynurenine pathway activity [206–208]. The upregulation of IDO1 and KMO in this inflammatory context increases the activity of the kynurenine pathway and favors the production of neurotoxic intermediates like QUIN. This response can further exacerbate systemic inflammation, indicating a more pathological role of the kynurenine pathway in obesity compared to its beneficial effects during exercise.

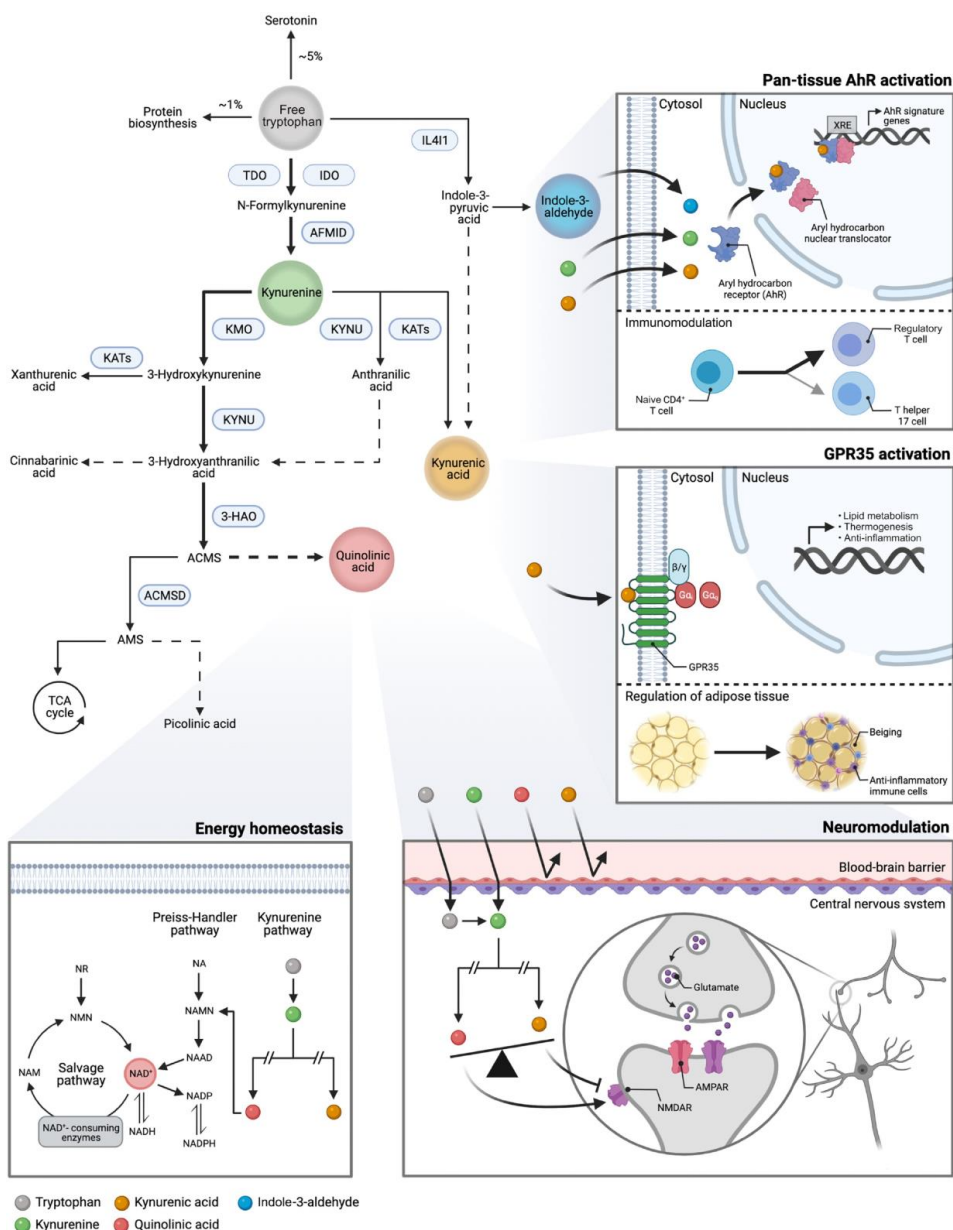


Figure 1.9. The kynurenine pathway and its physiological roles

A small portion of tryptophan is converted to serotonin, melatonin, or used in protein synthesis, but most is degraded via the kynurenine pathway. Under normal conditions, this pathway primarily produces quinolinic acid, which is used to generate NAD⁺, a crucial cofactor for cellular energy. Kynurenic acid, another metabolite in the pathway, plays key roles in various physiological processes. Notably, only tryptophan and kynurenine can cross the blood-brain barrier, while quinolinic acid and kynurenic acid cannot. The bold arrow indicates the primary metabolic route of tryptophan degradation, with unbroken arrows for enzymatic steps and broken arrows for spontaneous reactions. Reprinted with permission [197].

In summary, most tryptophan is metabolized via the kynurenine pathway, producing a range of bioactive metabolites. The balance between its branches is influenced by various stressors such as inflammation, exercise, and obesity. While the levels of KYNA and QUIN in the CNS have important implications for psychiatric disorders [209, 210], the kynurenine pathway metabolites have diverse physiological functions, with significant implications for both health and disease [197].

1.4.2 Kynurenine Metabolites and Their Biological Roles

The kynurenine pathway generates a variety of metabolites that influence both central and peripheral physiology. Among these, KYNA and QUIN are notable for their contrasting roles in inflammation, oxidative stress, and neuromodulation.

1.4.2.1 Kynurenic Acid (KYNA)

KYNA exhibits potent anti-inflammatory and antioxidant properties. It suppresses the expression and secretion of pro-inflammatory cytokines such as TNF- α in monocytes, IL-4 in invariant natural killer T (iNKT) cells, and IL-23 in dendritic cells [211]. In vivo, KYNA reduces TNF- α secretion in LPS-treated mice [212]. Additionally, KYNA scavenges reactive oxygen species (ROS), mitigating oxidative stress and preserving tissue integrity under inflammatory conditions [213].

KYNA's biological effects are mediated in part through its activation of G-protein coupled receptor 35 (GPR35) and the aryl hydrocarbon receptor (AhR) [205, 214]. GPR35 is expressed in various immune cells, including monocytes, mast cells, basophils, and iNKT cells, as well as in tissues such as the digestive tract, lung, and skeletal muscle. As a high-affinity ligand for GPR35, KYNA influences immune cell function by modulating signaling pathways involved in inflammation [214]. For example, KYNA inhibits N-type calcium channels in sympathetic neurons, reduces cAMP production, and downregulates inflammatory cascades, such as the PI3K/Akt and MAPK pathways [214]. These effects help limit inflammation and protect against excessive immune responses by modulating key signaling pathways, such as those involved in NF- κ B activation and cytokine release.

Similarly, KYNA's interaction with AhR regulates immune responses and prevents excessive inflammation. AhR is a ligand-activated transcription factor involved in immune response regulation [215–217]. For example, its activation induces

immune tolerance by promoting the differentiation of regulatory T cells and limiting the production of pro-inflammatory cytokines [214]. AhR-deficient mice demonstrate heightened inflammatory responses, underscoring the receptor's importance in immune homeostasis [218]. KYNA-mediated AhR activation also offers neuroprotection by countering excitotoxic damage induced by neurotoxic metabolites of the pathway, including QUIN.

In summary, KYNA plays a protective role by acting as a potent anti-inflammatory and antioxidant molecule. Through its interactions with GPR35 and AhR, KYNA modulates immune responses and helps maintain tissue homeostasis under inflammatory conditions. These multifaceted properties underscore KYNA's therapeutic potential in counteracting inflammation, oxidative stress, and neurodegeneration.

1.4.2.2 *Quinolinic Acid (QUIN)*

In stark contrast to KYNA, QUIN is a potent neurotoxin, exacerbating inflammation and oxidative stress [219]. Initial observations of QUIN's neurotoxic effects emerged when intracerebroventricular injections of QUIN in mice led to convulsions [220]. Further studies revealed that QUIN acts as a selective agonist at the N-methyl-D-aspartate (NMDA) receptor, triggering an influx of calcium into neurons and astrocytes, leading to excitotoxicity [219]. This is compounded by QUIN's ability to increase glutamate release while inhibiting its reuptake, further amplifying its neurotoxic effects [219]. Chronic exposure of human neurons to QUIN results in structural neuronal damage, including dendritic beading, microtubule disruption, and loss of organelles [221].

Beyond its direct neurotoxic effects, QUIN is a significant contributor to neuroinflammation. Intrastriatal administration of QUIN increases the expression of pro-inflammatory cytokines, such as TNF- α , further amplifying oxidative stress and neuronal damage [219]. During CNS inflammation, QUIN levels increase significantly, primarily due to enhanced synthesis by microglia in response to inflammatory stimuli such as IFN- γ . This elevated QUIN production is further supported by peripheral macrophages, which can produce up to 20- to 30-fold more QUIN than microglia [222]. The combined synthesis of QUIN by microglia and macrophages creates a positive feedback loop where inflammation stimulates QUIN production, which further amplifies inflammation and damages the blood-brain barrier (BBB) [197]. This permeability allows peripheral QUIN to enter the CNS, perpetuating the cycle of neuronal damage and inflammation.

Additionally, QUIN promotes oxidative stress through multiple mechanisms which can be independent of its activity at NMDARs. Through the NMDAR, QUIN induces calcium influx, leading to mitochondrial dysfunction and an increase in oxidative stress. Independent of NMDAR activation, QUIN interacts with iron to produce hydroxyl radicals through the Fenton reaction [223]. QUIN also activates nitric oxide synthase (NOS) in both astrocytes and neurons, amplifying free radical production and further exacerbating oxidative damage [224].

Together, QUIN is a multifaceted neurotoxin that exerts its harmful effects through neurotoxic, inflammatory, and oxidative pathways. By acting on NMDA receptors, amplifying inflammation, and promoting ROS production, QUIN contributes to a vicious cycle of neuronal damage and cell death. Its diverse mechanisms of action make it a key mediator in neurodegenerative and inflammatory conditions.

1.4.2.3 Kynurenine

Kynurenine serves as a central intermediate in the kynurenine pathway, linking upstream tryptophan metabolism to its downstream bioactive derivatives, such as KYNA and QUIN (Figure 1.9). Kynurenine has diverse physiological roles, including immune modulation, contributions to neurophysiology, and implications in disease pathogenesis [197].

The CNS receives approximately 60% of peripheral kynurenine via transport across the BBB, while the remaining is produced locally [225]. Under inflammatory conditions, increased BBB permeability can alter kynurenine flux, increasing its availability and exacerbating its neuroactive roles [197].

Like KYNA, kynurenine has immune-modulatory roles through the AhR [215]. Activation of AhR by kynurenine exhibits various effects depending on the cellular context. For example, AhR activation in tumor cells enhances the expression of genes that facilitate cell migration and metastasis [226]. Conversely, in immune cells, AhR activation dampens effector T-cell responses and fosters immune tolerance by targeting regulatory B cells and dendritic cells [227]. This immunosuppressive environment can be exploited by cancer cells to evade immune detection.

Exercise physiology provides another dimension to kynurenine's relevance, as the conversion of kynurenine to KYNA in skeletal muscle is hypothesized to underlie exercise-induced neuroprotective effects [199, 205]. Moreover, acute

endurance exercise has been shown to activate AhR in peripheral blood mononuclear cells, suggesting a mechanistic link between exercise-induced kynurenine metabolism and immune modulation [228]. This metabolic shift not only enhances KYNA production but may also contribute to an anti-inflammatory state, offering insights into the systemic benefits of physical activity.

1.4.3 Bioenergetic and Metabolic Aspects of The Kynurenine Pathway

With the multifaceted roles of kynurenine pathway metabolites, it is no surprise that the pathway has been implicated in several aspects of bioenergetics [198, 205, 229]. The kynurenine pathway contributes to the maintenance of cellular energy homeostasis by providing *de novo* synthesis of NAD⁺, a critical cofactor for enzymes involved in cellular energy metabolism and ATP production. NAD⁺ is also crucial for DNA repair, immune modulation, and transcriptional regulation [230]. Under physiological conditions, NAD⁺ is generated from tryptophan in most cell types, including neurons, astrocytes, and activated macrophages. In response to immune activation, macrophages increase ROS production, which can lead to cellular damage in chronic inflammatory conditions [231]. In these cases, kynurenine pathway metabolites help to compensate for the increased demand for NAD⁺, maintaining cellular energy balance despite the inflammatory stress [197].

Kynurenine pathway metabolites, particularly QUIN and KYNA, are well positioned to influence metabolic health through their modulation of NMDARs, where QUIN acts as an agonist and KYNA serves as an antagonist [232, 233]. While NMDARs are well-known for their role in neuronal function and excitotoxicity, they are also expressed on various cell types that contribute to whole-body metabolism. For example, in the pancreas, inhibition of NMDARs on β -cells enhances glucose-stimulated insulin secretion, potentially alleviating glucotoxicity and contributing to the beneficial effects of exercise in treating type-2 diabetes [234–236]. In rodents, activation of NMDARs exacerbates obesity-related pathogenesis, whereas NMDAR antagonism has been shown to alleviate this [237]. Notably, recent studies suggest that combining NMDAR antagonism with GLP-1 analogues could serve as a dual-action therapy for obesity treatment [238]. Therefore, the balance between NMDAR agonists and antagonists—such as QUIN and KYNA, respectively—may play a critical role in regulating whole-body energy metabolism and influencing metabolic health outcomes [200].

Exercise also regulates kynurenine pathway dynamics by promoting the conversion of kynurenine to KYNA through upregulation of KATs via a PGC-1 α -dependent mechanism [204, 239]. Kynurenine crosses the blood-brain barrier, while KYNA does not, enabling aerobically trained skeletal muscle to effectively detoxify neurotoxic kynurenine into neuroprotective KYNA. This strategy has been shown to reduce depressive-like symptoms in mice [199]. Additionally, KYNA increases adipose tissue energy expenditure and induces an anti-inflammatory gene expression profile in adipose tissue [205], highlighting its beneficial effects on metabolic health.

In summary, the kynurenine pathway plays a vital role in maintaining NAD⁺ balance, a key regulator of cellular energy metabolism. Given its effects on metabolic tissues such as muscle, adipose tissue, and pancreatic β -cells, the kynurenine pathway is emerging as a significant player in whole-body energy metabolism. Its ability to influence inflammatory responses, modulate NMDAR activity, and adapt to metabolic demands, positions it as a promising target for therapeutic interventions in metabolic diseases like obesity and type-2 diabetes. Continued research into the roles of kynurenine pathway metabolites could reveal novel strategies for improving metabolic health and managing related disorders.

1.4.4 Kynurenine Metabolites as Possible Regulators of Adipose-Sensory Nerve Crosstalk

Adipose tissue is richly innervated by sensory neurons, establishing a bidirectional communication network with the CNS. Although the precise biological roles of sensory neuron to adipose communication is still being uncovered, kynurenine pathway metabolites—particularly KYNA and QUIN—may act as critical modulators of adipose-sensory nerve crosstalk through their effects on NMDARs expressed on sensory neurons.

KYNA is an NMDAR antagonist with known neuroprotective activity in the CNS [240], while QUIN is an NMDAR agonist with strong excitotoxic properties [241–245]. NMDARs are ligand-gated ion channels traditionally associated with nociceptive transmission but are also expressed on sensory afferents that innervate peripheral tissues [169, 246–252]. When sensory neuron NMDARs are activated, they stimulate the local release of neuropeptides such as alpha-calcitonin gene-related peptide (CGRP α) and substance P (SP) [169, 170, 253].

Through these mechanisms, KYNA and QUIN could influence peripheral neuropeptide release and potentially regulate local tissue function.

Therapeutically, targeting peripherally restricted NMDAR ligands offers an intriguing strategy to modulate neuropeptide release without central side effects [158, 169]. For instance, QUIN, as an NMDAR agonist, does not cross the blood-brain barrier, making it a candidate for manipulating peripheral NMDAR activity. However, given that QUIN also serves as a precursor for NAD⁺, an essential molecule for cell viability. This dual role necessitates caution when considering strategies to manipulate QUIN levels, as it may have broader metabolic implications.

It is also important to note that sensory afferents not only release neuropeptides locally but also transmit signals centrally by releasing neurotransmitters at the presynaptic terminal in the spinal cord [170, 254]. Manipulating NMDAR activity at these central terminals could influence neurotransmitter release and alter transmission speed to the brain [255]. As kynurenine metabolites, including KYNA and QUIN, are present in both peripheral and central compartments, their potential regulatory roles may extend beyond local adipose tissue-neuron interactions to influence systemic and CNS-mediated processes.

1.5 Summary

The complex network of interactions between different organ systems is fundamental to maintaining homeostasis, allowing organisms to integrate neural and molecular signals and adapt to physiological demands. In this thesis thus far, we have discussed key aspects of inter-organ communication, focusing on adipose tissue, skeletal muscle, and the kynurenine pathway of tryptophan metabolism. We explored adipose tissue's central role in whole-body energy metabolism, emphasizing both the importance of adipogenesis for healthy tissue expansion and the critical influence of its neural innervation. We then discussed skeletal muscle regeneration, highlighting the intricate interactions among immune cells, muscle fibers, and satellite cells that are essential for effective repair and functional recovery. Finally, we discussed how the bioactive metabolites produced by the kynurenine pathway of tryptophan degradation possess diverse biological functions and are uniquely positioned to influence whole body metabolic health. By further understanding the mechanisms of inter-organ communication explored throughout this thesis, we can better define their implications for health and disease and identify new therapeutic opportunities.

2 Research Aims

The overarching theme of this thesis is to investigate inter-organ communication within the contexts of adipose tissue and skeletal muscle biology. Both tissues are critical regulators of systemic energy homeostasis and are influenced by diverse signaling pathways, including those mediated by neural, metabolic, and immune cues. This work aims to deepen our understanding of these pathways and their implications for health and disease. The specific aims are:

Paper I: Constitutive loss of kynurenine-3-monooxygenase changes circulating kynurenine metabolites without affecting systemic energy metabolism.

The kynurenine pathway of tryptophan degradation is influenced by metabolic stressors, with exercise increasing circulating kynurenine acid levels, while obesity is associated with elevated quinolinic acid. This study aimed to investigate the metabolic consequences of constitutive loss of kynurenine-3-monooxygenase (KMO), which shifts kynurenine metabolism away from quinolinic acid towards kynurenine acid production. The findings clarify the role of chronic KMO inhibition in metabolic regulation and provide valuable insights into ongoing development of KMO inhibitors as potential therapies.

Paper II: Zfp697 is an RNA-binding protein that regulates skeletal muscle inflammation and remodeling.

Skeletal muscle recovery from atrophy and injury involves complex remodeling processes driven by interactions among muscle fibers, immune cells, and satellite cells. Recently, our group identified Zfp697, a gene induced during skeletal muscle recovery following atrophy. The aim of this study was to investigate how Zfp697 regulates regenerative responses in skeletal muscle. Understanding Zfp697's role in muscle remodeling provides insights into therapeutic strategies to enhance recovery from muscle damage.

Paper III: Sensory neuron-derived alpha-calcitonin gene-related peptide controls adipogenesis.

The sensory innervation of adipose tissue mediates part of the bidirectional communication between the brain and adipose tissue; however, the local release of neuropeptides by sensory neurons and their effects on adipose tissue remain poorly understood. Alpha-calcitonin gene-related peptide (CGRP α) is a sensory-

neuron-derived neuropeptide and is elevated in models of obesity. Here, we aimed to evaluate the impact of CGRP α on adipogenesis and its potential role in adipose tissue remodeling. Our findings provide novel insights into the local effects of sensory neuropeptides on adipose tissue biology, with potential implications for CGRP α -targeted therapies, which are already used for migraine treatment and may influence broader metabolic effects.

3 Results and Discussion

3.1 Paper I: Constitutive loss of kynurenine-3-monooxygenase changes circulating kynurenine metabolites without affecting systemic energy metabolism.

Tryptophan degradation primarily occurs via the ubiquitous kynurenine pathway. This pathway is a critical metabolic route that supports the de novo synthesis of NAD⁺ but also generates bioactive metabolites with diverse regulatory roles in immune, neurological, and bioenergetic processes [197]. Among these metabolites, kynurenic acid (KYNA) and quinolinic acid (QUIN) are particularly well-studied due to their well-established roles in the central nervous system [199, 204]. However, growing evidence suggests that their roles extend to whole body energy metabolism [205]. This is potentially mediated through their opposing interactions with NMDARs, where QUIN acts as an agonist and KYNA as an antagonist [232, 233]. Given the critical role of NMDARs in regulating energy metabolism—including glucose-stimulated insulin secretion [234–236] and responses to high-fat diets in mice [237]—the balance of NMDAR agonists and antagonists in peripheral tissues, such as QUIN and KYNA, respectively, may significantly influence systemic metabolic outcomes.

The potential role of these metabolites in energy metabolism is further emphasized by the kynurenine pathway's sensitivity to metabolic stressors. For example, circulating KYNA levels rise following exercise, whereas obesity is associated with elevated QUIN production [206–208, 256]. These observations suggest that the pathway is a key mediator of metabolic adaptation, with KYNA and QUIN potentially acting as regulators of whole body energy metabolism. This led us to hypothesize that the genetic deletion of kynurenine 3-monooxygenase (KMO)—leading to increased circulating KYNA and decreased QUIN—would manifest as differences in whole body energy metabolism.

Generation and Validation of the KMO^{NULL} Model

The production of QUIN is contingent upon the activity of KMO, a rate-limiting enzyme that directs the kynurenine pathway toward QUIN synthesis. Conversely, the inhibition or loss of KMO activity shifts the pathway toward KYNA production. This makes KMO a valuable target for manipulating the balance of these metabolites. Here, we used a knockout-first strategy to eliminate *Kmo* gene expression (KMO^{NULL}), which we validated by measuring the expression and

protein levels on both chow and high-fat diet conditions. Notably, while knockout efficiency can vary depending on context [257], our approach achieved robust suppression across dietary conditions. This provided a stable and reliable model to examine the systemic metabolic effects of altered kynurenine pathway metabolites.

Consistent with previous studies, KMO^{NULL} mice exhibited a profound increase in circulating KYNA levels and a marked reduction in QUIN [258–262]. These biochemical changes confirm the expected metabolic shifts resulting from KMO deletion, providing a model for studying the systemic effects of high KYNA and low QUIN levels.

KMO^{NULL} Mice on a Chow or High-Fat Diet Exhibit Comparable Metabolic Phenotypes Compared to Controls

To evaluate the impact of altered kynurenine metabolites on whole-body energy metabolism, we performed comprehensive metabolic assessments, including glucose tolerance tests, body composition analysis, glucose-stimulated insulin secretion, and indirect calorimetry. Surprisingly, despite profound changes in circulating metabolite levels, KMO^{NULL} mice displayed no significant changes in body weight, glucose handling, insulin secretion, or energy expenditure. Challenging these mice with a 15-wk high-fat diet yielded similar results: KMO^{NULL} and wildtype controls (KMO^{WT}) mice exhibited comparable metabolic phenotypes under obesogenic conditions.

Influence of Genetic Background on Metabolic Phenotypes

The above findings contrast earlier studies reporting a ~10% increase in body weight and higher fasting blood glucose levels in KMO^{NULL} mice [258, 259]. One potential explanation for the discrepancies between our results and previous reports lies in the genetic background of the mice used. While our study used mice on a C57BL/6N background, these earlier studies used mice on a C57BL/6J background. Although these strains differ only by a single letter in name, they have several known genetic differences. Notably, C57BL/6J mice possess a mutation in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene, which affects mitochondrial function and predisposes them to high-fat diet-induced metabolic disease [263]. For example, C57BL/6J mice gain more weight and exhibit greater glucose intolerance than their C57BL/6N counterparts when challenged with a high-fat diet [264].

To investigate the potential influence of the *Nnt* mutation, we crossed KMO^{NULL} mice with C57BL/6J wild-type mice and selected for the presence of the mutation. As such, this cohort resulted in KMO^{NULL} and KMO^{WT} mice all with an *Nnt* mutation. However, even under these conditions, KMO^{NULL} and KMO^{WT} had similar body weights and glucose tolerance responses. While this suggests that the *Nnt* mutation alone does not account for the observed discrepancies between studies, other genetic differences between the strains may play a role [265, 266]. Further backcrossing onto a pure C57BL/6J background will be essential to fully elucidate these effects.

Chronically Elevated KYNA and Adipose Tissue Biology

Given the known thermogenic and immunomodulatory effects of KYNA in adipose tissue [205], we hypothesized that chronically elevated KYNA levels in KMO^{NULL} mice would enhance energy expenditure or alter adipose tissue transcriptomes. However, transcriptomic analysis of iWAT revealed no significant differences beyond the deletion of *Kmo* itself. This suggests that the chronic changes in circulating metabolites, including KYNA, does not replicate the effects of acute or exercise-induced KYNA increases [205, 267–269].

One explanation may lie in the kinetics of KYNA elevation. Acute increases, such as those induced by exercise, may elicit distinct biological responses compared to sustained elevation. Indeed, KYNA has been shown to enhance beta-adrenergic signaling in primary adipocytes with repeated treatments, while a single dose has no effect [205]. Additionally, chronic KYNA elevation may lead to receptor desensitization or internalization, a process well documented for many G protein-coupled receptors. These findings highlight the complexity of KYNA signaling and the need for further research into its temporal dynamics and receptor-mediated effects.

Alternatively, the effects of KYNA may be influenced by its receptor pharmacology. To date, four receptors have been proposed to interact with KYNA: NMDARs, AhR, GPR35, and $\alpha 7$ nAChRs [197]. KYNA acts as an agonist at GPR35, influencing energy balance and inflammatory responses [205, 270]. Conversely, it serves as an antagonist at NMDARs, modulating glutamatergic neurotransmission [240]. The role of KYNA at $\alpha 7$ nAChRs is more nuanced; while some studies suggest antagonistic effects [271], others find no significant interaction [272]. Additionally, KYNA activates AhR, linking tryptophan metabolism to immune regulation [215–217]. The diverse expression of these

receptors across various cell types and their distinct biological activities makes it challenging to predict the outcomes of fluctuating KYNA levels.

Broader Implications of Altered Kynurenine Metabolites

While this project was initially driven by the hypothesis that KYNA and QUIN are key modulators of energy metabolism, our findings reveal that KMO^{NULL} mice exhibit a markedly altered circulating metabolome. These changes extend beyond KYNA and QUIN, with notable increases in anthranilic acid and reductions in 3-hydroxyanthranilic acid. The anthranilic branch of the kynurenine pathway provides an alternate route to produce QUIN independent of KMO. However, the next metabolite after anthranilic acid, 3-hydroxyanthranilic acid, was reduced, suggesting that the conversion of anthranilic acid to 3-hydroxyanthranilic acid is not a major pathway.

Moreover, the interplay between metabolites of the kynurenine pathway warrants further investigation, as their combined effects may modulate systemic energy metabolism in ways that individual metabolite studies cannot capture. For instance, while KYNA has thermogenic properties, kynurenine exacerbates HFD-induced obesity and insulin resistance [273]. The simultaneous elevation of KYNA and kynurenine in KMO^{NULL} mice may result in opposing metabolic effects, effectively neutralizing their individual impacts. These findings highlight the complexity of the kynurenine pathway and suggest that systemic metabolic outcomes may depend on the combined influence of its metabolites rather than the actions of any single compound.

Conclusion

Our findings demonstrate that chronic alterations in kynurenine pathway metabolites via genetic deletion of *Kmo* do not significantly impact whole body energy metabolism in mice on a C57BL/6N background. These results challenge previous reports and emphasize the need for careful consideration of genetic background and metabolite kinetics in preclinical research. By integrating metabolic, biochemical, and transcriptomic data, this study provides a comprehensive foundation for future investigations into the kynurenine pathway's role in systemic metabolism and its therapeutic potential.

3.2 Paper II: Zfp697 is an RNA-binding protein that regulates skeletal muscle inflammation and remodeling.

Skeletal muscle comprises approximately 40% of total body mass in healthy individuals, playing a central role in locomotion, energy metabolism, and thermoregulation. Beyond these roles, muscle mass and function are directly linked to overall health and inversely associated with mortality, underscoring its importance to quality of life [172]. Remarkably, skeletal muscle can regenerate and adapt to changes in use or disuse, altering its size, metabolism, and fiber composition. This regenerative capacity is crucial for recovery from atrophy and injury; however, its failure can result in fibrosis and irreversible loss of function, as seen in muscular dystrophies and aging-related sarcopenia [172, 186].

Understanding the complex interplay of immune cells, extracellular matrix remodeling, and protein turnover in muscle regeneration is key to advancing therapies for injuries, intense exercise recovery, and genetic muscle diseases.

Zfp697 Expression Is Induced in Skeletal Muscle During Regeneration and Remodeling

Using a well-established mouse model of hindlimb unloading and reloading, we aimed to uncover novel regulators of muscle regeneration. This model recapitulates the progression of muscle atrophy due to disuse, followed by compensatory hypertrophy and regeneration during reloading. Through transcriptomic analysis, we identified the previously uncharacterized zinc finger protein 697 (*Zfp697* in mice and *ZNF697* in humans) as significantly upregulated during the early stages of muscle reloading [274]. *Zfp697* expression was transient, peaking during the regenerative phase before subsiding as muscle structure and function normalized. This expression pattern was corroborated in independent models of skeletal muscle remodeling, including cardiotoxin-induced injury and intense physical exercise, underscoring *Zfp697*'s conserved role across contexts.

Our findings were further reinforced by publicly available human datasets, which revealed conserved *Zfp697* expression in human skeletal muscle. Notably, *ZFP697* was enriched in regenerative myonuclei populations, including those associated with denervated or dystrophic muscle fibers, suggesting a conserved role across species and underscoring its potential translational relevance. However, human data often lack the temporal resolution and experimental

control achievable in animal models, emphasizing the value of integrating diverse models to capture the complexity of muscle remodeling.

The hindlimb unloading and reloading model remains a cornerstone in our approach due to its ability to capture atrophy, hypertrophy, and regeneration within the same system. Despite its utility, its limitations must be acknowledged, including the lack of chronic pathological features, such as those seen in cachexia or sarcopenia, and potential systemic effects like altered vascular or neural inputs. These factors may obscure muscle-specific mechanisms of regeneration, highlighting the importance of this consideration. By leveraging complementary approaches however, we demonstrated the transient upregulation of Zfp697 across regenerative contexts, suggesting its critical role in skeletal muscle remodeling.

Zfp697 Regulates Inflammatory and Interferon Signaling Pathways in Skeletal Muscle

Skeletal muscle regeneration requires a well-coordinated immune response, characterized by early pro-inflammatory signals to recruit immune cells and activate satellite cells, followed by resolution of inflammation to facilitate tissue repair [186]. Our data demonstrate that Zfp697 plays a central role in these processes by activating a broad inflammatory gene program in myotubes and intact muscle tissue.

Functional analyses of cultured myotubes revealed that overexpression of Zfp697 induced chemokine production, upregulated interferon-stimulated genes, and enriched interferon- α and - γ signaling pathways. Conversely, Zfp697 knockdown impaired the myotube response to interferon- γ , highlighting its role as a mediator of interferon signaling in muscle cells. Interestingly, Zfp697 knockdown not only dampened basal interferon signaling but also hindered the transcriptional response to injury-associated stressors. These findings align with clinical studies showing that variants in ZFP697 are linked to altered interferon- β responses in multiple sclerosis patients [275], suggesting a broader role for Zfp697 in immune modulation.

The observed link between Zfp697 and interferon signaling pathways raises intriguing questions about its broader implications in immune modulation. Given that interferon responses play a dual role in both promoting and resolving inflammation, Zfp697 may act as a critical modulator of this balance. Investigating its interaction with other immune regulators, such as NF- κ B or STAT

proteins, could provide deeper insights into its function. Moreover, whether Zfp697's role extends to non-muscle tissues or systemic immune responses warrants further exploration.

Clinically, these findings highlight Zfp697 as a potential therapeutic target for enhancing muscle regeneration or modulating immune responses in disease contexts. However, translating these insights into therapeutic strategies poses challenges, including the need to finely tune its activity to avoid excessive or insufficient immune responses. Further understanding of how Zfp697 expression and activity are regulated could reveal additional therapeutic targets for modulating its levels in different pathological conditions. Future studies using advanced genetic tools, such as tissue-specific knockouts or CRISPR-mediated gene editing, will be invaluable in delineating the precise mechanistic contributions of Zfp697 to muscle and immune biology.

Zfp697 Is Essential for Muscle Regeneration In Vivo

To assess the physiological importance of Zfp697, we generated myofiber-specific Zfp697 knockout (mKO) mice. Under basal conditions and during hindlimb unloading, mKO mice displayed no significant differences in muscle mass, fiber size, or gene expression compared to control mice. However, upon reloading, mKO mice exhibited profound defects in muscle regeneration. Across three different injury–recovery paradigms—hindlimb reloading, downhill running, and cardiotoxin-induced injury—mKO mice were unable to recover muscle mass, strength, or normal gait parameters. At the molecular level, these deficiencies were associated with impaired activation of genes involved in inflammation, ECM remodeling, angiogenesis, and cell proliferation.

Further investigation revealed a marked reduction in satellite cell activation and fibro-adipogenic precursor (FAP) dynamics in mKO mice. Satellite cells, essential for muscle repair, failed to proliferate and differentiate effectively in the absence of Zfp697. Similarly, FAPs, which transiently promote ECM remodeling and support satellite cell function, exhibited reduced activation, compromising the overall regenerative response. This striking loss of regenerative capacity underscores the critical role of Zfp697 in muscle repair. However, given that damaged myofiber-derived factors (DMDFs), such as metabolic enzymes like GAPDH, have been shown to promote satellite cell activation [188], this impairment is perhaps not entirely unexpected. One possibility is that Zfp697 influences the release or activity of these factors, thereby regulating the

regenerative response. Alternatively, Zfp697 itself may function as a DMDF, acting directly on satellite cells to facilitate their activation and proliferation. Supporting this idea, our deconvolution analysis of RNA-seq data revealed that Zfp697 mKO mice failed to expand key regenerative cell populations, including FAPs and satellite cells, during the early stages of reloading-induced regeneration. This failure was further reflected in reduced numbers of Pax7-positive satellite cells and fewer proliferative Ki67-positive cells in mKO muscle. Collectively, these findings suggest that Zfp697 is a crucial component of the myofiber-intrinsic response to injury and plays a key role in coordinating the broader regenerative program.

Mechanistic Insights into Zfp697 Function

Zfp697 is a member of the zinc finger protein (ZFP) family, known for its diverse roles in transcriptional regulation, RNA binding, and protein-protein interactions [276]. Although initially hypothesized to act primarily as a transcription factor, our data suggest that Zfp697 functions primarily as an RNA-binding protein in skeletal muscle. Enhanced crosslinking and immunoprecipitation (eCLIP) analysis revealed that Zfp697 preferentially binds processed mRNAs and miRNAs, including miR-206, a muscle-specific microRNA (myomiR) involved in muscle regeneration and pathology. Its strong affinity for miRNAs positions Zfp697 as a key modulator of post-transcriptional regulation, influencing pathways critical for muscle repair and regeneration.

Interestingly, Zfp697 also exhibited enrichment in retrotransposable element-derived RNAs, which are potent activators of interferon responses. This finding suggests a dual role for Zfp697, not only in modulating immune signaling but also in maintaining RNA homeostasis during cellular stress. Given the well-documented involvement of interferon responses in muscle repair and aging, Zfp697 emerges as a key integrator of both transcriptional and post-transcriptional regulatory networks.

Conclusion

Our findings position Zfp697 as a pivotal regulator of muscle regeneration, orchestrating processes at the intersection of immune signaling, ECM remodeling, and RNA homeostasis. Acting as a molecular switch, Zfp697 facilitates the transition from the pro-inflammatory phase of injury repair to tissue regeneration, preventing prolonged activation that could lead to fibrosis or chronic inflammation. By modulating chemokine expression and interferon-

stimulated genes, Zfp697 recruits and activates macrophages while maintaining the balance between constructive and destructive ECM remodeling, critical for successful regeneration. Additionally, Zfp697's RNA-binding capacity adds complexity to its role, ensuring RNA homeostasis during cellular stress by regulating miRNAs and retrotransposon-derived RNAs. This dual functionality not only supports efficient resolution of inflammation but also protects against chronic inflammatory states that impair regeneration. Together, these findings establish Zfp697 as a central player in the coordination of immune, cellular, and molecular processes required for effective muscle repair, with potential implications for understanding and treating muscle-related pathologies.

3.3 Paper III: Sensory neuron-derived alpha-calcitonin gene-related peptide controls adipogenesis.

Sensory nerve activation leads to the localized release of neuropeptides, which are known to play key roles in various physiological processes. However, the effects of these locally released neuropeptides, particularly within adipose tissue, are not well understood. In this study, we investigate the role of alpha-calcitonin gene-related peptide (CGRP α), a neuropeptide released from sensory neurons, in adipose tissue biology. Growing evidence suggests that CGRP α is involved in energy homeostasis, with models of obesity showing elevated circulating CGRP α levels [147–150]. Furthermore, anti-CGRP α -targeted therapies, established as frontline treatments for migraine, have shown potential weight-modulating effects [277–279], highlighting the importance of understanding CGRP α 's role in metabolic regulation.

CGRP α Inhibits Adipocyte Differentiation In Vitro

To investigate the effect CGRP α on preadipocytes, we treated primary murine adipocyte cultures derived from the iWAT of male mice. Our in vitro experiments showed that CGRP α significantly inhibits adipocyte differentiation, as shown by reduced adipocyte formation and dysregulated expression of key adipogenic transcription factors. These findings align with previous studies that have demonstrated CGRP α 's role in differentiation in various cell types [280–286]. Importantly, pre-treatment with a CGRP α receptor antagonist reversed these effects, indicating a receptor-mediated mechanism. Additionally, we identified a critical time window during early differentiation where CGRP α exerted its effect.

RNA sequencing of differentiating preadipocytes treated with CGRP α revealed a fibro-inflammatory gene signature, along with evidence of ECM remodeling. Notably, we observed upregulation of inflammatory markers, including cytokines, in response to CGRP α . This raises the possibility that CGRP α triggers a cascade where it acts on cells to initiate inflammation, which in turn promotes cytokine release. The released cytokines could then feedback onto the cells, further exacerbating the inhibition of differentiation. This feedback loop suggests that CGRP α 's effects might not be solely limited to direct signaling but could involve an inflammatory component that amplifies the inhibition of adipocyte differentiation.

Interestingly, the highest differentially expressed gene in our RNA-sequencing dataset was *Nos2*, which encodes nitric oxide synthase, an enzyme involved in

nitric oxide (NO) production. Studies have shown that NO inhibits adipocyte differentiation and promotes a pro-fibrogenic response [287]. However, the role of NO in adipogenesis remains controversial [288]. In any case, the interaction between sensory neurons and adipose tissue is an area that has not been extensively characterized, particularly regarding how nerve endings communicate with adipocytes. It remains unclear whether these neurons form true synapses or junctions with adipocytes or if they rely on signal diffusion or propagation to transmit information [289]. The potential for secondary signaling events, such as the release of cytokines or NO, to propagate sensory-derived signals from the sensory neurons to other cells within the adipose tissue remains a testable hypothesis.

In Vivo Knockdown of CGRP α Reveals Complex Effects on Adipocyte Size Distribution

To test the in vivo effects of CGRP α on adipocyte differentiation, we specifically targeted the knockdown of CGRP α in sensory neurons innervating the inguinal iWAT. Based on our in vitro findings, we hypothesized that reducing CGRP α would relieve the inhibition of adipogenesis and result in smaller adipocytes. However, contrary to our expectations, analysis of adipocyte size distribution showed an increase in the mean and median adipocyte size. These results suggest that the effects of CGRP α on adipose tissue in vivo are more complex than initially anticipated.

One possible explanation for this discrepancy is that, while we selected a strategy to modify CGRP α while minimizing compensatory sympathetic signalling, some residual sympathetic activity may still have occurred. Although we did not specifically measure sympathetic signals, this could have influenced the outcome. Additionally, while our primary focus was on adipogenesis, CGRP α is known to affect mature adipocytes as well [153, 290–294]. The interaction between CGRP α and various cell types in adipose tissue could therefore involve complementary or even opposing roles.

Furthermore, the model we used did not include a specific stressor to induce differentiation. At room temperature, the absence of such a stimulus may have been insufficient to trigger adipogenesis. For instance, cold exposure has been shown to increase the number of new adipocytes in iWAT [48]. Therefore, an experiment involving cold exposure and knockdown or overexpression of CGRP α

would perhaps yield different results, potentially providing further insights into the role of CGRP α in adipogenesis under stress conditions.

The observed increase in adipocyte size in the CGRP α knockdown model also raises the question of whether adipocyte size alone is an adequate measure of CGRP α 's impact on adipose tissue. A more direct assessment of adipogenesis could be achieved using models such as the adipochaser mouse [48], which labels new versus old adipocytes. This approach would provide a more precise measurement of CGRP α 's effects on adipocyte differentiation, offering a clearer picture of its role in adipose tissue remodeling beyond adipocyte size distribution.

Possible Sexual Dimorphism in CGRP α Sensitivity

An intriguing consideration is the potential for sex differences in response to CGRP α . CGRP α has a well-established role in migraine, a condition that disproportionately affects women, suggesting that females may be more sensitive to CGRP α than males [295]. If this pattern extends to adipose tissue, it raises the possibility that the effects of CGRP α on adipogenesis and adipose tissue remodeling could differ between sexes. Given that females represent the largest group using CGRP α -targeting migraine therapies, understanding how these medications influence adipose tissue biology is particularly important. Future investigations should explore whether these sex-specific differences in CGRP α sensitivity influence adipose tissue biology, as this could have important implications for understanding metabolic regulation and therapeutic strategies.

Conclusion

This study highlights the effects of CGRP α on adipose tissue biology, particularly its inhibitory influence on adipocyte differentiation in vitro and its influence on adipose tissue characteristics in vivo. Our findings underscore the complexity of neuropeptide signaling within adipose tissue, suggesting that CGRP α 's actions are mediated by a combination of direct receptor interactions, inflammatory cascades, and potential secondary signaling pathways. Moreover, the possibility of sex-specific responses and tissue-specific compensatory mechanisms emphasizes the need for further research to unravel the diverse roles of CGRP α in metabolic regulation. These insights not only enhance our understanding of the sensory nervous system's contribution to adipose tissue dynamics but also hold implications for the development of targeted therapies addressing metabolic disorders.

4 Concluding Remarks and Future Directions

The survival and adaptability of multicellular organisms rely on the effective coordination of their internal systems through complex networks of inter-organ communication. This communication, facilitated by the nervous system and the exchange of signaling molecules, helps maintain physiological balance and enables dynamic responses to fluctuations in nutrient availability, energy demand, and stress. Disruptions in these networks can lead to metabolic imbalances, impacting growth, immunity, and overall health. Thus, understanding inter-organ communication is key to uncovering the mechanisms behind health and disease.

This thesis explored the complex role of inter-organ communication, focusing on three key topics: the kynurenine pathway of tryptophan degradation and its impact on whole body energy metabolism, the role of the zinc finger protein Zfp697 in skeletal muscle inflammation and remodeling, and the effects of sensory-derived CGRP α on adipose tissue. Through these studies, this thesis highlighted how metabolic pathways and signaling mechanisms across different tissues integrate to maintain homeostasis and how disruptions in these processes contribute to metabolic dysfunction. By uncovering the molecular and cellular underpinnings of these interactions, this work provides valuable insights into whole-body metabolism, muscle regeneration and inflammation, and adipose tissue remodeling, offering potential avenues for therapeutic strategies across metabolic and musculoskeletal disorders.

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6 References

1. Galenus, C., *De usu partium corporis humani, libri XVII.* apud Gulielmum Rouillium.
2. Bernard, C., *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux.* Paris: J. B. Baillière, 1878: p. 564.
3. Cannon, W.B., *Physiological regulation of normal states: some tentative postulates concerning biological homeostatics.* Ses Amis, ses Colleges, ses Eleves, 1926.
4. Cannon, W.B., *Organization for physiological homeostasis.* Physiological reviews, 1929. **9**(3): p. 399–431.
5. Katagiri, H., *Inter-organ communication involved in metabolic regulation at the whole-body level.* Inflamm Regen, 2023. **43**(1): p. 60.
6. Castillo-Armengol, J., L. Fajas, and I.C. Lopez-Mejia, *Inter-organ communication: a gatekeeper for metabolic health.* EMBO Rep, 2019. **20**(9): p. e47903.
7. Tokizane, K. and S.I. Imai, *Inter-organ communication is a critical machinery to regulate metabolism and aging.* Trends Endocrinol Metab, 2024.
8. Cui, L., et al., *Major depressive disorder: hypothesis, mechanism, prevention and treatment.* Signal Transduct Target Ther, 2024. **9**(1): p. 30.
9. Herrlich, A., E. Kefaloyianni, and S. Rose-John, *Mechanisms of interorgan crosstalk in health and disease.* FEBS Lett, 2022. **596**(5): p. 529–533.
10. Zhang, Y., P.B. Alexander, and X.F. Wang, *TGF- β Family Signaling in the Control of Cell Proliferation and Survival.* Cold Spring Harb Perspect Biol, 2017. **9**(4).
11. Droujinine, I.A. and N. Perrimon, *Interorgan Communication Pathways in Physiology: Focus on Drosophila.* Annu Rev Genet, 2016. **50**: p. 539–570.
12. Hu, S., Y. Hu, and W. Yan, *Extracellular vesicle-mediated interorgan communication in metabolic diseases.* Trends Endocrinol Metab, 2023. **34**(9): p. 571–582.
13. Scheele, C. and C. Wolfum, *Brown Adipose Crosstalk in Tissue Plasticity and Human Metabolism.* Endocr Rev, 2020. **41**(1): p. 53–65.
14. Clemente-Suarez, V.J., et al., *The Role of Adipokines in Health and Disease.* Biomedicines, 2023. **11**(5).
15. Munoz-Canoves, P., et al., *Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword?* FEBS J, 2013. **280**(17): p. 4131–48.
16. Preidis, G.A., K.H. Kim, and D.D. Moore, *Nutrient-sensing nuclear receptors PPAR α and FXR control liver energy balance.* J Clin Invest, 2017. **127**(4): p. 1193–1201.
17. Riera, C.E., *Wiring the Brain for Wellness: Sensory Integration in Feeding and Thermogenesis: A Report on Research Supported by Pathway to Stop Diabetes.* Diabetes, 2024. **73**(3): p. 338–347.
18. Roh, E. and M.S. Kim, *Brain Regulation of Energy Metabolism.* Endocrinol Metab (Seoul), 2016. **31**(4): p. 519–524.
19. Ahmed, B., R. Sultana, and M.W. Greene, *Adipose tissue and insulin resistance in obese.* Biomed Pharmacother, 2021. **137**: p. 111315.
20. Zhang, Y., et al., *Positional cloning of the mouse obese gene and its human homologue.* Nature, 1994. **372**(6505): p. 425–432.
21. Myers, M.G., M.A. Cowley, and H. Munzberg, *Mechanisms of leptin action and leptin resistance.* Annu Rev Physiol, 2008. **70**: p. 537–56.
22. Sakers, A., et al., *Adipose-tissue plasticity in health and disease.* Cell, 2022. **185**(3): p. 419–446.
23. Smorlesi, A., et al., *The adipose organ: white-brown adipocyte plasticity and metabolic inflammation.* Obes Rev, 2012. **13 Suppl 2**: p. 83–96.
24. Nisoli, E. and S. Cinti, *What defines a cell type? Perspectives from adipocyte biology.* Int J Obes (Lond), 2024.
25. Efremova, A., et al., *A large proportion of mediastinal and perirenal visceral fat of Siberian adult people is formed by UCPI immunoreactive multilocular and paucilocular adipocytes.* J Physiol Biochem, 2020. **76**(2): p. 185–192.
26. Giordano, A., et al., *White, brown and pink adipocytes: the extraordinary plasticity of the adipose organ.* Eur J Endocrinol, 2014. **170**(5): p. R159–71.
27. Rosen, E.D. and B.M. Spiegelman, *What we talk about when we talk about fat.* Cell, 2014. **156**(1–2): p. 20–44.
28. Scheja, L. and J. Heeren, *The endocrine function of adipose tissues in health and cardiometabolic disease.* Nat Rev Endocrinol, 2019. **15**(9): p. 507–524.
29. Crewe, C., Y.A. An, and P.E. Scherer, *The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis.* J Clin Invest, 2017. **127**(1): p. 74–82.
30. Cedikova, M., et al., *Mitochondria in White, Brown, and Beige Adipocytes.* Stem Cells Int, 2016. **2016**: p. 6067349.
31. Morrison, S.F., K. Nakamura, and C.J. Madden, *Central control of thermogenesis in mammals.* Exp Physiol, 2008. **93**(7): p. 773–97.
32. Marlatt, K.L., K.Y. Chen, and E. Ravussin, *Is activation of human brown adipose tissue a viable target for weight management?* Am J

- Physiol Regul Integr Comp Physiol, 2018. **315**(3): p. R479–R483.
33. Scheele, C. and S. Nielsen, *Metabolic regulation and the anti-obesity perspectives of human brown fat*. Redox Biol, 2017. **12**: p. 770–775.
 34. Altshuler-Keylin, S., et al., *Beige Adipocyte Maintenance Is Regulated by Autophagy-Induced Mitochondrial Clearance*. Cell Metab, 2016. **24**(3): p. 402–419.
 35. Schirinzi, V., et al., *Browning of Adipocytes: A Potential Therapeutic Approach to Obesity*. Nutrients, 2023. **15**(9).
 36. Morroni, M., et al., *Reversible transdifferentiation of secretory epithelial cells into adipocytes in the mammary gland*. Proceedings of the National Academy of Sciences, 2004. **101**(48): p. 16801–16806.
 37. Fried, S.K., M.J. Lee, and K. Karastergiou, *Shaping fat distribution: New insights into the molecular determinants of depot- and sex-dependent adipose biology*. Obesity (Silver Spring), 2015. **23**(7): p. 1345–52.
 38. Ziegler, A.K. and C. Scheele, *Human adipose depots' diverse functions and dysregulations during cardiometabolic disease*. NPJ Metab Health Dis, 2024. **2**(1): p. 34.
 39. Zwick, R.K., et al., *Anatomical, Physiological, and Functional Diversity of Adipose Tissue*. Cell Metab, 2018. **27**(1): p. 68–83.
 40. Tchkonja, T., et al., *Mechanisms and metabolic implications of regional differences among fat depots*. Cell Metab, 2013. **17**(5): p. 644–656.
 41. Borgeson, E., J. Boucher, and C.E. Hagberg, *Of mice and men: Pinpointing species differences in adipose tissue biology*. Front Cell Dev Biol, 2022. **10**: p. 1003118.
 42. Lee, M.J., Y. Wu, and S.K. Fried, *Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications*. Mol Aspects Med, 2013. **34**(1): p. 1–11.
 43. Lefebvre, A.-M., et al., *Depot-Specific Differences in Adipose Tissue Gene Expression in Lean and Obese Subjects*. Diabetes, 1998. **47**(1): p. 98–103.
 44. Macotela, Y., et al., *Intrinsic differences in adipocyte precursor cells from different white fat depots*. Diabetes, 2012. **61**(7): p. 1691–9.
 45. Vidal, H., *Gene expression in visceral and subcutaneous adipose tissues*. Ann Med, 2001. **33**(8): p. 547–55.
 46. Mathur, N., et al., *Human visceral and subcutaneous adipose stem and progenitor cells retain depot-specific adipogenic properties during obesity*. Front Cell Dev Biol, 2022. **10**: p. 983899.
 47. Tchkonja, T., et al., *Fat depot origin affects adipogenesis in primary cultured and cloned human preadipocytes*. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2002. **282**(5): p. R1286–R1296.
 48. Wang, Q.A., et al., *Tracking adipogenesis during white adipose tissue development, expansion and regeneration*. Nat Med, 2013. **19**(10): p. 1338–44.
 49. Bilal, M., et al., *Fate of adipocyte progenitors during adipogenesis in mice fed a high-fat diet*. Mol Metab, 2021. **54**: p. 101328.
 50. Wernstedt Asterholm, I., et al., *Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling*. Cell Metab, 2014. **20**(1): p. 103–18.
 51. Stephens, J.M. and P.H. Pekala, *Transcriptional repression of the C/EBP- α and GLUT4 genes in 3T3-L1 adipocytes by tumor necrosis factor- α . Regulations is coordinate and independent of protein synthesis*. Journal of Biological Chemistry, 1992. **267**(19): p. 13580–13584.
 52. Kern, P.A., et al., *The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase*. The Journal of Clinical Investigation, 1995. **95**(5): p. 2111–2119.
 53. Fontana, L., et al., *Visceral fat adipokine secretion is associated with systemic inflammation in obese humans*. Diabetes, 2007. **56**(4): p. 1010–3.
 54. Cartier, A., et al., *Visceral obesity and plasma glucose-insulin homeostasis: contributions of interleukin-6 and tumor necrosis factor- α in men*. J Clin Endocrinol Metab, 2008. **93**(5): p. 1931–8.
 55. Kahn, D., et al., *Exploring Visceral and Subcutaneous Adipose Tissue Secretomes in Human Obesity: Implications for Metabolic Disease*. Endocrinology, 2022. **163**(11).
 56. Ziegler, A.K., et al., *An anti-inflammatory phenotype in visceral adipose tissue of old lean mice, augmented by exercise*. Sci Rep, 2019. **9**(1): p. 12069.
 57. Shao, M., et al., *De novo adipocyte differentiation from Pdgfr β + preadipocytes protects against pathologic visceral adipose expansion in obesity*. Nature Communications, 2018. **9**(1).
 58. Palani, N.P., et al., *Adipogenic and SWAT cells separate from a common progenitor in human brown and white adipose depots*. Nat Metab, 2023. **5**(6): p. 996–1013.
 59. Arner, E., et al., *Adipocyte turnover: relevance to human adipose tissue morphology*. Diabetes, 2010. **59**(1): p. 105–9.
 60. Rosen, E.D. and O.A. MacDougald, *Adipocyte differentiation from the inside out*. Nat Rev Mol Cell Biol, 2006. **7**(12): p. 885–96.
 61. Cristancho, A.G. and M.A. Lazar, *Forming functional fat: a growing understanding of adipocyte differentiation*. Nat Rev Mol Cell Biol, 2011. **12**(11): p. 722–34.

62. Farmer, S.R., *Transcriptional control of adipocyte formation*. Cell Metab, 2006. **4**(4): p. 263–73.
63. Siersbaek, R. and S. Mandrup, *Transcriptional networks controlling adipocyte differentiation*. Cold Spring Harb Symp Quant Biol, 2011. **76**: p. 247–55.
64. Morrison, R.F. and S.R. Farmer, *Role of PPARgamma in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18(INK4c) and p21(Waf1/Cip1), during adipogenesis*. J Biol Chem, 1999. **274**(24): p. 17088–97.
65. Klingelhuber, F., et al., *A spatiotemporal proteomic map of human adipogenesis*. Nature Metabolism, 2024. **6**(5): p. 861–879.
66. Ghaben, A.L. and P.E. Scherer, *Adipogenesis and metabolic health*. Nat Rev Mol Cell Biol, 2019. **20**(4): p. 242–258.
67. Takada, I., A.P. Kouzmenko, and S. Kato, *Wnt and PPARgamma signaling in osteoblastogenesis and adipogenesis*. Nat Rev Rheumatol, 2009. **5**(8): p. 442–7.
68. Hepler, C. and R.K. Gupta, *The expanding problem of adipose depot remodeling and postnatal adipocyte progenitor recruitment*. Mol Cell Endocrinol, 2017. **445**: p. 95–108.
69. Tang, Q.-Q., T.C. Otto, and M.D. Lane, *Mitotic clonal expansion: A synchronous process required for adipogenesis*. Proceedings of the National Academy of Sciences, 2003. **100**(1): p. 44–49.
70. Logan, C.Y. and R. Nusse, *The Wnt signaling pathway in development and disease*. Annu. Rev. Cell Dev. Biol., 2004. **20**: p. 781–810.
71. Okamura, M., et al., *COUP-TFII acts downstream of Wnt/ β -catenin signal to silence PPAR γ gene expression and repress adipogenesis*. Proceedings of the National Academy of Sciences, 2009. **106**(14): p. 5819–5824.
72. Rawadi, G., et al., *BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop*. J Bone Miner Res, 2003. **18**(10): p. 1842–53.
73. Ross, S.E., et al., *Inhibition of Adipogenesis by Wnt Signaling*. Science, 2000. **289**(5481): p. 950–953.
74. Kanazawa, A., et al., *Wnt5b partially inhibits canonical Wnt/ β -catenin signaling pathway and promotes adipogenesis in 3T3-L1 preadipocytes*. Biochem Biophys Res Commun, 2005. **330**(2): p. 505–10.
75. Takada, I., et al., *A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR- γ transactivation*. Nat Cell Biol, 2007. **9**(11): p. 1273–85.
76. Fuster, J.J., et al., *Noncanonical Wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction independent of adipose tissue expansion*. Diabetes, 2015. **64**(4): p. 1235–48.
77. Zamani, N. and C.W. Brown, *Emerging roles for the transforming growth factor- β superfamily in regulating adiposity and energy expenditure*. Endocr Rev, 2011. **32**(3): p. 387–403.
78. Choy, L., J. Skillington, and R. Derynck, *Roles of Autocrine TGF- β Receptor and Smad Signaling in Adipocyte Differentiation*. Journal of Cell Biology, 2000. **149**(3): p. 667–682.
79. Tang, Q.-Q., T.C. Otto, and M.D. Lane, *Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage*. Proceedings of the National Academy of Sciences, 2004. **101**(26): p. 9607–9611.
80. Schulz, T.J. and Y.H. Tseng, *Emerging role of bone morphogenetic proteins in adipogenesis and energy metabolism*. Cytokine Growth Factor Rev, 2009. **20**(5–6): p. 523–31.
81. Choy, L. and R. Derynck, *Transforming growth factor- β inhibits adipocyte differentiation by Smad3 interacting with CCAAT/enhancer-binding protein (C/EBP) and repressing C/EBP transactivation function*. J Biol Chem, 2003. **278**(11): p. 9609–19.
82. Hammarstedt, A., et al., *WISP2 regulates preadipocyte commitment and PPAR γ activation by BMP4*. Proceedings of the National Academy of Sciences, 2013. **110**(7): p. 2563–2568.
83. Gustafson, B., et al., *BMP4 and BMP Antagonists Regulate Human White and Beige Adipogenesis*. Diabetes, 2015. **64**(5): p. 1670–81.
84. Alessi, M.C., et al., *Plasminogen activator inhibitor 1, transforming growth factor- β 1, and BMI are closely associated in human adipose tissue during morbid obesity*. Diabetes, 2000. **49**(8): p. 1374–1380.
85. Yadav, H., et al., *Protection from Obesity and Diabetes by Blockade of TGF- β /Smad3 Signaling*. Cell Metabolism, 2011. **14**(1): p. 67–79.
86. Bernlohr, D.A., et al., *Evidence for an increase in transcription of specific mRNAs during differentiation of 3T3-L1 preadipocytes*. Journal of Biological Chemistry, 1985. **260**(9): p. 5563–5567.
87. Zhao, M.L., et al., *Molecular Competition in G1 Controls When Cells Simultaneously Commit to Terminally Differentiate and Exit the Cell Cycle*. Cell Rep, 2020. **31**(1): p. 107769.
88. Patel, Y.M. and M.D. Lane, *Mitotic Clonal Expansion during Preadipocyte Differentiation: Calcipain-mediated Turnover of p27*. Journal of Biological Chemistry, 2000. **275**(23): p. 17653–17660.
89. Tang, Q.-Q., et al., *Sequential phosphorylation of CCAAT enhancer-binding protein β by MAPK and glycogen synthase kinase 3 β is required for adipogenesis*. Proceedings of the National Academy of Sciences, 2005. **102**(28): p. 9766–9771.
90. Park, B.H., L. Qiang, and S.R. Farmer, *Phosphorylation of C/EBP β at a consensus extracellular signal-regulated kinase/glycogen*

- synthase kinase 3 site is required for the induction of adiponectin gene expression during the differentiation of mouse fibroblasts into adipocytes. *Mol Cell Biol*, 2004. **24**(19): p. 8671–80.
91. Tang, Q.-Q., T.C. Otto, and M.D. Lane, CCAAT/enhancer-binding protein β is required for mitotic clonal expansion during adipogenesis. *Proceedings of the National Academy of Sciences*, 2003. **100**(3): p. 850–855.
92. Tang, Q.Q. and M.D. Lane, Activation and centromeric localization of CCAAT/enhancer-binding proteins during the mitotic clonal expansion of adipocyte differentiation. *Genes Dev*, 1999. **13**(17): p. 2231–41.
93. Mischoulon, D., et al., Growth-dependent inhibition of CCAAT enhancer-binding protein (C/EBP α) gene expression during hepatocyte proliferation in the regenerating liver and in culture. *Molecular and Cellular Biology*, 1992. **12**(6): p. 2553–2560.
94. Timchenko, N.A., et al., CCAAT/enhancer-binding protein α (C/EBP α) inhibits cell proliferation through the p21 (WAF-1/CIP-1/SDI-1) protein. *Genes & Development*, 1996. **10**(7): p. 804–815.
95. Wang, H., et al., C/EBP α Arrests Cell Proliferation through Direct Inhibition of Cdk2 and Cdk4. *Molecular Cell*, 2001. **8**(4): p. 817–828.
96. Lin, F.T., et al., A 30-kDa alternative translation product of the CCAAT/enhancer binding protein α message: transcriptional activator lacking antimitotic activity. *Proceedings of the National Academy of Sciences*, 1993. **90**(20): p. 9606–9610.
97. Altiock, S., M. Xu, and B.M. Spiegelman, PPAR γ induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. *Genes & Development*, 1997. **11**(15): p. 1987–1998.
98. Harris, T.E., et al., CCAAT/Enhancer-binding Protein- α Cooperates with p21 to Inhibit Cyclin-dependent Kinase-2 Activity and Induces Growth Arrest Independent of DNA Binding. *Journal of Biological Chemistry*, 2001. **276**(31): p. 29200–29209.
99. Tang, Q.Q., J.W. Zhang, and M. Daniel Lane, Sequential gene promoter interactions of C/EBP β , C/EBP α , and PPAR γ during adipogenesis. *Biochem Biophys Res Commun*, 2004. **319**(1): p. 235–9.
100. Yeh, W.C., et al., Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev*, 1995. **9**(2): p. 168–81.
101. Rosen, E.D., et al., PPAR γ Is Required for the Differentiation of Adipose Tissue In Vivo and In Vitro. *Molecular Cell*, 1999. **4**(4): p. 611–617.
102. Rosen, E.D., et al., C/EBP α induces adipogenesis through PPAR γ : a unified pathway. *Genes Dev*, 2002. **16**(1): p. 22–6.
103. Fain, J.N., D.S. Tichansky, and A.K. Madan, Transforming growth factor β release by human adipose tissue is enhanced in obesity. *Metabolism*, 2005. **54**(11): p. 1546–51.
104. Blaszkiewicz, M., et al., The Importance of Peripheral Nerves in Adipose Tissue for the Regulation of Energy Balance. *Biology*, 2019. **8**(1): p. 10.
105. Guilherme, A., et al., Molecular pathways linking adipose innervation to insulin action in obesity and diabetes mellitus. *Nature Reviews Endocrinology*, 2019. **15**(4): p. 207–225.
106. Bartness, T.J., et al., Neural innervation of white adipose tissue and the control of lipolysis. *Frontiers in neuroendocrinology*, 2014. **35**(4): p. 473–493.
107. Bartness, T.J., et al., Brain–adipose tissue cross talk. *Proceedings of the Nutrition Society*, 2005. **64**(1): p. 53–64.
108. Giordano, A., et al., White adipose tissue lacks significant vagal innervation and immunohistochemical evidence of parasympathetic innervation. *Am J Physiol Regul Integr Comp Physiol*, 2006. **291**(5): p. R1243–55.
109. Puente-Ruiz, S.C. and A. Jais, Reciprocal signaling between adipose tissue depots and the central nervous system. *Front Cell Dev Biol*, 2022. **10**: p. 979251.
110. Bartness, T.J., C.H. Vaughan, and C.K. Song, Sympathetic and sensory innervation of brown adipose tissue. *Int J Obes (Lond)*, 2010. **34 Suppl 1**(O 1): p. S36–42.
111. Bartness, T.J., et al., Sensory and sympathetic nervous system control of white adipose tissue lipolysis. *Mol Cell Endocrinol*, 2010. **318**(1–2): p. 34–43.
112. Bowers, R.R., et al., Sympathetic innervation of white adipose tissue and its regulation of fat cell number. *American Journal of Physiology–Regulatory, Integrative and Comparative Physiology*, 2004. **286**(6): p. R1167–R1175.
113. Foster, M.T. and T.J. Bartness, Sympathetic but not sensory denervation stimulates white adipocyte proliferation. *Am J Physiol Regul Integr Comp Physiol*, 2006. **291**(6): p. R1630–7.
114. Dogiel, A., Die sensiblen Nervenendigungen im Herzen und in den Blutgefäßen der Säugethiere. *Archiv für mikroskopische Anatomie*, 1898. **52**(1): p. 44–70.
115. Youngstrom, T.G. and T.J. Bartness, Catecholaminergic innervation of white adipose tissue in Siberian hamsters. *American Journal of Physiology–Regulatory, Integrative and Comparative Physiology*, 1995. **268**(3): p. R744–R751.
116. Bartness, T.J. and M. Bamshad, Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. *American Journal of Physiology–Regulatory, Integrative and*

- Comparative Physiology, 1998. **275**(5): p. R1399–R1411.
117. Willows, J.W., et al., *Visualization and Analysis of Whole Depot Adipose Tissue Neural Innervation*. 2021.
118. Collins, S., W. Cao, and J. Robidoux, *Learning new tricks from old dogs: beta-adrenergic receptors teach new lessons on firing up adipose tissue metabolism*. *Mol Endocrinol*, 2004. **18**(9): p. 2123–31.
119. Woo, Y.C., et al., *Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives*. *Clin Endocrinol (Oxf)*, 2013. **78**(4): p. 489–96.
120. Magro, B.S. and D.P.M. Dias, *Brown and beige adipose tissue: New therapeutic targets for metabolic disorders*. *Health Sciences Review*, 2024. **10**.
121. Jones, D., et al., *Norepinephrine inhibits rat pre-adipocyte proliferation*. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity*, 1992. **16**(5): p. 349–354.
122. Shi, H., et al., *Sensory or sympathetic white adipose tissue denervation differentially affects depot growth and cellularity*. *American Journal of Physiology–Regulatory, Integrative and Comparative Physiology*, 2005. **288**(4): p. R1028–R1037.
123. Cousin, B., et al., *Local sympathetic denervation of white adipose tissue in rats induces preadipocyte proliferation without noticeable changes in metabolism*. *Endocrinology*, 1993. **133**(5): p. 2255–2262.
124. Zhu, Y., et al., *Sympathetic neuropeptide Y protects from obesity by sustaining thermogenic fat*. *Nature*, 2024. **634**(8032): p. 243–250.
125. Fredholm, B.B., *Chapter 3 – Nervous control of circulation and metabolism in white adipose tissue*, in *New Perspectives in Adipose Tissue*, A. Cryer and R.L.R. Van, Editors. 1985, Butterworth–Heinemann. p. 45–64.
126. Fishman, R.B. and J. Dark, *Sensory innervation of white adipose tissue*. *American Journal of Physiology–Regulatory, Integrative and Comparative Physiology*, 1987. **253**(6): p. R942–R944.
127. Giordano, A., et al., *Tyrosine hydroxylase, neuropeptide Y, substance P, calcitonin gene-related peptide and vasoactive intestinal peptide in nerves of rat periovarian adipose tissue: an immunohistochemical and ultrastructural investigation*. *Journal of Neurocytology*, 1996. **25**(1): p. 125–136.
128. Shi, H. and T.J. Bartness, *White adipose tissue sensory nerve denervation mimics lipectomy-induced compensatory increases in adiposity*. *Am J Physiol Regul Integr Comp Physiol*, 2005. **289**(2): p. R514–R520.
129. Frei, I.C., et al., *Adipose mTORC2 is essential for sensory innervation in white adipose tissue and whole-body energy homeostasis*. *Mol Metab*, 2022. **65**: p. 101580.
130. Wang, Y., et al., *The role of somatosensory innervation of adipose tissues*. *Nature*, 2022. **609**(7927): p. 569–574.
131. Nijijima, A., *Reflex effects from leptin sensors in the white adipose tissue of the epididymis to the efferent activity of the sympathetic and vagus nerve in the rat*. *Neuroscience Letters*, 1999. **262**(2): p. 125–128.
132. Nijijima, A., *Afferent signals from leptin sensors in the white adipose tissue of the epididymis, and their reflex effect in the rat*. *Journal of the Autonomic Nervous System*, 1998. **73**(1): p. 19–25.
133. Considine, R.V., et al., *Serum immunoreactive-leptin concentrations in normal-weight and obese humans*. *New England Journal of Medicine*, 1996. **334**(5): p. 292–295.
134. Shi, Z., et al., *Sympathetic activation by chemical stimulation of white adipose tissues in rats*. *J Appl Physiol* (1985), 2012. **112**(6): p. 1008–14.
135. Tanida, M., et al., *Leptin injection into white adipose tissue elevates renal sympathetic nerve activity dose-dependently through the afferent nerves pathway in rats*. *Neuroscience Letters*, 2000. **293**(2): p. 107–110.
136. Garrettson, J.T., et al., *Lipolysis sensation by white fat afferent nerves triggers brown fat thermogenesis*. *Mol Metab*, 2016. **5**(8): p. 626–634.
137. Murphy, K.T., et al., *Leptin-sensitive sensory nerves innervate white fat*. *Am J Physiol Endocrinol Metab*, 2013. **304**(12): p. E1338–47.
138. Shi, H., R.R. Bowers, and T.J. Bartness, *Norepinephrine turnover in brown and white adipose tissue after partial lipectomy*. *Physiol Behav*, 2004. **81**(3): p. 535–42.
139. Nguyen, N.L.T., B. Xue, and T.J. Bartness, *Sensory denervation of inguinal white fat modifies sympathetic outflow to white and brown fat in Siberian hamsters*. *Physiol Behav*, 2018. **190**: p. 28–33.
140. Youngstrom, T.G. and T.J. Bartness, *White adipose tissue sympathetic nervous system denervation increases fat pad mass and fat cell number*. *American Journal of Physiology–Regulatory, Integrative and Comparative Physiology*, 1998. **275**(5): p. R1488–R1493.
141. Riera, C.E., et al., *TRPV1 pain receptors regulate longevity and metabolism by neuropeptide signaling*. *Cell*, 2014. **157**(5): p. 1023–36.
142. Makwana, K., et al., *Sensory neurons expressing calcitonin gene-related peptide alpha regulate adaptive thermogenesis and diet-induced obesity*. *Mol Metab*, 2021. **45**: p. 101161.
143. Passini, F.S., et al., *Piezo2 in sensory neurons regulates systemic and adipose tissue metabolism*. *Cell Metab*, 2025.

144. Saria, A., et al., *Simultaneous release of several tachykinins and calcitonin gene-related peptide from rat spinal cord slices*. *Neurosci Lett*, 1986. **63**(3): p. 310-4.
145. Maggi, C.A. and A. Meli, *The sensory-efferent function of capsaicin-sensitive sensory neurons*. *General Pharmacology: The Vascular System*, 1988. **19**(1): p. 1-43.
146. Russell, F.A., et al., *Calcitonin gene-related peptide: physiology and pathophysiology*. *Physiol Rev*, 2014. **94**(4): p. 1099-142.
147. Halloran, J., et al., *Monoclonal therapy against calcitonin gene-related peptide lowers hyperglycemia and adiposity in type 2 diabetes mouse models*. *Metabol Open*, 2020. **8**: p. 100060.
148. Zelissen, P.M.J., et al., *Calcitonin gene-related peptide in human obesity*. *Peptides*, 1991. **12**(4): p. 861-863.
149. Gram, D.X., et al., *Plasma calcitonin gene-related peptide is increased prior to obesity, and sensory nerve desensitization by capsaicin improves oral glucose tolerance in obese Zucker rats*. *Eur J Endocrinol*, 2005. **153**(6): p. 963-9.
150. Tanaka, H., et al., *Enhanced insulin secretion and sensitization in diabetic mice on chronic treatment with a transient receptor potential vanilloid 1 antagonist*. *Life Sci*, 2011. **88**(11-12): p. 559-63.
151. Lönnqvist, F., et al., *Overexpression of the obese (ob) gene in adipose tissue of human obese subjects*. *Nature Medicine*, 1995. **1**(9): p. 950-953.
152. Amer, P. and M. Ryden, *Fatty Acids, Obesity and Insulin Resistance*. *Obes Facts*, 2015. **8**(2): p. 147-55.
153. Chatzipanteli, K., et al., *Calcitonin gene-related peptide is an adipose-tissue neuropeptide with lipolytic actions*. *Endocrinology and Metabolism, Supplement*, 1996. **3**(4): p. 235-242.
154. Gram, D.X., et al., *Sensory nerve desensitization by resiniferatoxin improves glucose tolerance and increases insulin secretion in Zucker Diabetic Fatty rats and is associated with reduced plasma activity of dipeptidyl peptidase IV*. *Eur J Pharmacol*, 2005. **509**(2-3): p. 211-7.
155. Walker, C.S., et al., *Mice lacking the neuropeptide alpha-calcitonin gene-related peptide are protected against diet-induced obesity*. *Endocrinology*, 2010. **151**(9): p. 4257-69.
156. Gazelius, B., et al., *Vasodilatory effects and coexistence of calcitonin gene-related peptide (CGRP) and substance P in sensory nerves of cat dental pulp*. *Acta Physiologica Scandinavica*, 1987. **130**(1): p. 33-40.
157. Fu, J., et al., *Substance P is associated with the development of obesity, chronic inflammation and type 2 diabetes mellitus*. *Exp Clin Endocrinol Diabetes*, 2011. **119**(3): p. 177-81.
158. Karagiannides, I., et al., *Induction of colitis causes inflammatory responses in fat depots: Evidence for substance P pathways in human mesenteric preadipocytes*. *Proceedings of the National Academy of Sciences*, 2006. **103**(13): p. 5207-5212.
159. Miegueu, P., et al., *Substance P decreases fat storage and increases adipocytokine production in 3T3-L1 adipocytes*. *Am J Physiol Gastrointest Liver Physiol*, 2013. **304**(4): p. G420-7.
160. Gross, K., et al., *Substance P promotes expansion of human mesenteric preadipocytes through proliferative and antiapoptotic pathways*. *Am J Physiol Gastrointest Liver Physiol*, 2009. **296**(5): p. G1012-9.
161. Karagiannides, I., et al., *Substance P (SP)-neurokinin-1 receptor (NK-1R) alters adipose tissue responses to high-fat diet and insulin action*. *Endocrinology*, 2011. **152**(6): p. 2197-205.
162. Dubon, M.J., Y. Byeon, and K.S. Park, *Substance P enhances the activation of AMPK and cellular lipid accumulation in 3T3-L1 cells in response to high levels of glucose*. *Mol Med Rep*, 2015. **12**(6): p. 8048-54.
163. Karagiannides, I., et al., *Substance P as a novel anti-obesity target*. *Gastroenterology*, 2008. **134**(3): p. 747-55.
164. Wiesenfeld-Hallin, Z., et al., *Immunoreactive calcitonin gene-related peptide and substance P coexist in sensory neurons to the spinal cord and interact in spinal behavioral responses of the rat*. *Neurosci Lett*, 1984. **52**(1-2): p. 199-204.
165. Lundberg, J.M., et al., *Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin*. *Eur J Pharmacol*, 1985. **108**(3): p. 315-9.
166. Ju, G., et al., *Primary sensory neurons of the rat showing calcitonin gene-related peptide immunoreactivity and their relation to substance P-, somatostatin-, galanin-, vasoactive intestinal polypeptide- and cholecystokinin-immunoreactive ganglion cells*. *Cell Tissue Res*, 1987. **247**(2): p. 417-31.
167. Franco-Cereceda, A., et al., *Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin*. *Peptides*, 1987. **8**(2): p. 399-410.
168. Hökfelt, T., et al., *Coexistence of peptides with classical neurotransmitters*. *Experientia*, 1987. **43**(7): p. 768-780.
169. McRoberts, J.A., et al., *Role of peripheral N-methyl-D-aspartate (NMDA) receptors in visceral nociception in rats*. *Gastroenterology*, 2001. **120**(7): p. 1737-1748.
170. Liu, H., P.W. Mantyh, and A.I. Basbaum, *NMDA-receptor regulation of substance P release from*

- primary afferent nociceptors. *Nature*, 1997. **386**(6626): p. 721-724.
171. Gamse, R. and A. Saria, *Potentialiation of tachykinin-induced plasma protein extravasation by calcitonin gene-related peptide*. *Eur J Pharmacol*, 1985. **114**(1): p. 61-6.
172. Frontera, W.R. and J. Ochala, *Skeletal muscle: a brief review of structure and function*. *Calcif Tissue Int*, 2015. **96**(3): p. 183-95.
173. Schiaffino, S. and C. Reggiani, *Fiber types in mammalian skeletal muscles*. *Physiol Rev*, 2011. **91**(4): p. 1447-531.
174. Baskin, K.K., B.R. Winders, and E.N. Olson, *Muscle as a "mediator" of systemic metabolism*. *Cell Metab*, 2015. **21**(2): p. 237-248.
175. Thiebaud, D., et al., *The Effect of Graded Doses of Insulin on Total Glucose Uptake, Glucose Oxidation, and Glucose Storage in Man*. *Diabetes*, 1982. **31**(11): p. 957-963.
176. DeFronzo, R.A. and D. Tripathy, *Skeletal muscle insulin resistance is the primary defect in type 2 diabetes*. *Diabetes Care*, 2009. **32 Suppl 2**(Suppl 2): p. S157-63.
177. Goodman, C.A., et al., *Novel insights into the regulation of skeletal muscle protein synthesis as revealed by a new nonradioactive in vivo technique*. *FASEB J*, 2011. **25**(3): p. 1028-39.
178. Wu, Z., et al., *Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1 α* . *Cell*, 1999. **98**(1): p. 115-124.
179. Scheele, C., S. Nielsen, and B.K. Pedersen, *ROS and myokines promote muscle adaptation to exercise*. *Trends Endocrinol Metab*, 2009. **20**(3): p. 95-9.
180. Matthews, V.B., et al., *Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase*. *Diabetologia*, 2009. **52**(7): p. 1409-18.
181. Yin, L., et al., *Skeletal muscle atrophy: From mechanisms to treatments*. *Pharmacol Res*, 2021. **172**: p. 105807.
182. Sandri, M., et al., *Foxo Transcription Factors Induce the Atrophy-Related Ubiquitin Ligase Atrogin-1 and Cause Skeletal Muscle Atrophy*. *Cell*, 2004. **117**(3): p. 399-412.
183. Bodine, S.C., et al., *Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo*. *Nature Cell Biology*, 2001. **3**(11): p. 1014-1019.
184. Mammucari, C., et al., *FoxO3 controls autophagy in skeletal muscle in vivo*. *Cell Metab*, 2007. **6**(6): p. 458-71.
185. Sartori, R., V. Romanello, and M. Sandri, *Mechanisms of muscle atrophy and hypertrophy: implications in health and disease*. *Nat Commun*, 2021. **12**(1): p. 330.
186. Forcina, L., M. Cosentino, and A. Musaro, *Mechanisms Regulating Muscle Regeneration: Insights into the Interrelated and Time-Dependent Phases of Tissue Healing*. *Cells*, 2020. **9**(5).
187. Yang, W. and P. Hu, *Skeletal muscle regeneration is modulated by inflammation*. *J Orthop Translat*, 2018. **13**: p. 25-32.
188. Tsuchiya, Y., et al., *Damaged Myofiber-Derived Metabolic Enzymes Act as Activators of Muscle Satellite Cells*. *Stem Cell Reports*, 2020. **15**(4): p. 926-940.
189. Brack, A.S., et al., *A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis*. *Cell Stem Cell*, 2008. **2**(1): p. 50-9.
190. Leikina, E., et al., *Myomaker and Myomerger Work Independently to Control Distinct Steps of Membrane Remodeling during Myoblast Fusion*. *Dev Cell*, 2018. **46**(6): p. 767-780 e7.
191. Verma, M., et al., *Muscle Satellite Cell Cross-Talk with a Vascular Niche Maintains Quiescence via VEGF and Notch Signaling*. *Cell Stem Cell*, 2018. **23**(4): p. 530-543 e9.
192. Tisdale, M.J., *Mechanisms of cancer cachexia*. *Physiol Rev*, 2009. **89**(2): p. 381-410.
193. Reza, M.M., et al., *Irisin is a pro-myogenic factor that induces skeletal muscle hypertrophy and rescues denervation-induced atrophy*. *Nat Commun*, 2017. **8**(1): p. 1104.
194. Long, A.M., et al., *Extracellular matrix contribution to disease progression and dysfunction in myopathy*. *Am J Physiol Cell Physiol*, 2023. **325**(5): p. C1244-C1251.
195. Wang, J.H., et al., *Adeno-associated virus as a delivery vector for gene therapy of human diseases*. *Signal Transduct Target Ther*, 2024. **9**(1): p. 78.
196. Porcu, C., G. Dobrowolny, and B.M. Scicchitano, *Exploring the Role of Extracellular Vesicles in Skeletal Muscle Regeneration*. *Int J Mol Sci*, 2024. **25**(11).
197. Joisten, N., et al., *The kynurenine pathway in chronic diseases: a compensatory mechanism or a driving force?* *Trends in Molecular Medicine*, 2021.
198. Cervenka, I., L.Z. Agudelo, and J.L. Ruas, *Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health*. *Science*, 2017. **357**(6349).
199. Agudelo, L.Z., et al., *Skeletal muscle PGC-1 α modulates kynurenine metabolism and mediates resilience to stress-induced depression*. *Cell*, 2014. **159**(1): p. 33-45.
200. Dumont, K.D., et al., *Constitutive loss of kynurenine-3-monooxygenase changes circulating kynurenine metabolites without affecting systemic energy metabolism*. *Am J*

- Physiol Endocrinol Metab, 2025. **328**(2): p. E274–E285.
201. Sadik, A., et al., *IL4I1 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression*. Cell, 2020. **182**(5): p. 1252–1270 e34.
202. Alberati-Giani, D., et al., *Regulation of the kynurenine metabolic pathway by interferon- γ in murine cloned macrophages and microglial cells*. J Neurochem, 1996. **66**(3): p. 996–1004.
203. Baumgartner, R., M.J. Forteza, and D.F.J. Ketelhuth, *The interplay between cytokines and the Kynurenine pathway in inflammation and atherosclerosis*. Cytokine, 2019. **122**: p. 154148.
204. Schlittler, M., et al., *Endurance exercise increases skeletal muscle kynurenine aminotransferases and plasma kynurenic acid in humans*. Am J Physiol Cell Physiol, 2016. **310**(10): p. C836–40.
205. Agudelo, L.Z., et al., *Kynurenic Acid and Gpr35 Regulate Adipose Tissue Energy Homeostasis and Inflammation*. Cell Metab, 2018. **27**(2): p. 378–392 e5.
206. Farup, P.G., et al., *The Kynurenine Pathway in Healthy Subjects and Subjects with Obesity, Depression and Chronic Obstructive Pulmonary Disease*. Pharmaceuticals (Basel), 2023. **16**(3).
207. Favennec, M., et al., *The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation*. Obesity, 2015. **23**(10): p. 2066–2074.
208. Wang, M.E., et al., *Adiposity and plasma concentrations of kynurenine pathway metabolites and traditional markers of inflammation*. Obesity Research & Clinical Practice, 2023.
209. Erhardt, S., et al., *The kynurenic acid hypothesis of schizophrenia*. Physiol Behav, 2007. **92**(1–2): p. 203–9.
210. Marx, W., et al., *The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies*. Mol Psychiatry, 2021. **26**(8): p. 4158–4178.
211. Salimi Elizei, S., et al., *Kynurenic acid downregulates IL-17/IL-23 axis in vitro*. Mol Cell Biochem, 2017. **431**(1–2): p. 55–65.
212. Moroni, F., et al., *Kynurenic acid actions in brain and periphery*. International Congress Series, 2007. **1304**: p. 305–313.
213. Lugo-Huitron, R., et al., *On the antioxidant properties of kynurenic acid: free radical scavenging activity and inhibition of oxidative stress*. Neurotoxicol Teratol, 2011. **33**(5): p. 538–47.
214. Wirthgen, E., et al., *Kynurenic Acid: The Janus-Faced Role of an Immunomodulatory Tryptophan Metabolite and Its Link to Pathological Conditions*. Front Immunol, 2017. **8**: p. 1957.
215. Mezrich, J.D., et al., *An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells*. J Immunol, 2010. **185**(6): p. 3190–8.
216. DiNatale, B.C., et al., *Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling*. Toxicol Sci, 2010. **115**(1): p. 89–97.
217. Seok, S.H., et al., *Trace derivatives of kynurenine potently activate the aryl hydrocarbon receptor (AHR)*. J Biol Chem, 2018. **293**(6): p. 1994–2005.
218. Sekine, H., et al., *Hypersensitivity of aryl hydrocarbon receptor-deficient mice to lipopolysaccharide-induced septic shock*. Mol Cell Biol, 2009. **29**(24): p. 6391–400.
219. Lugo-Huitrón, R., et al., *Quinolinic acid: an endogenous neurotoxin with multiple targets*. Oxidative medicine and cellular longevity, 2013. **2013**.
220. Lapin, I.P., *Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice*. Journal of Neural Transmission, 1978. **42**(1): p. 37–43.
221. Rahman, A., et al., *The excitotoxin quinolinic acid induces tau phosphorylation in human neurons*. PLoS One, 2009. **4**(7): p. e6344.
222. Guillemín, G.J., et al., *Expression of the kynurenine pathway enzymes in human microglia and macrophages*. Adv Exp Med Biol, 2003. **527**: p. 105–12.
223. Iwahashi, H., H. Kawamori, and K. Fukushima, *Quinolinic acid, α -picolinic acid, fusaric acid, and 2,6-pyridinedicarboxylic acid enhance the Fenton reaction in phosphate buffer*. Chemico-Biological Interactions, 1999. **118**(3): p. 201–215.
224. Braidy, N., et al., *Neuroprotective effects of naturally occurring polyphenols on quinolinic acid-induced excitotoxicity in human neurons*. FEBS J, 2010. **277**(2): p. 368–82.
225. Savitz, J., *The kynurenine pathway: a finger in every pie*. Mol Psychiatry, 2020. **25**(1): p. 131–147.
226. D'Amato, N.C., et al., *A TDO2-AhR signaling axis facilitates anoikis resistance and metastasis in triple-negative breast cancer*. Cancer Res, 2015. **75**(21): p. 4651–64.
227. Opitz, C.A., et al., *An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor*. Nature, 2011. **478**(7368): p. 197–203.
228. Joisten, N., et al., *Acute exercise activates the AHR in peripheral blood mononuclear cells in an intensity-dependent manner*. Am J Physiol Cell Physiol, 2024. **327**(2): p. C438–C445.
229. Martin, K.S., M. Azzolini, and J. Lira Ruas, *The kynurenine connection: how exercise shifts muscle tryptophan metabolism and affects energy homeostasis, the immune system, and the brain*. Am J Physiol Cell Physiol, 2020. **318**(5): p. C818–C830.

230. Braidy, N., et al., *Role of Nicotinamide Adenine Dinucleotide and Related Precursors as Therapeutic Targets for Age-Related Degenerative Diseases: Rationale, Biochemistry, Pharmacokinetics, and Outcomes*. Antioxid Redox Signal, 2019. **30**(2): p. 251–294.
231. Braidy, N. and R. Grant, *Kynurenine pathway metabolism and neuroinflammatory disease*. Neural Regen Res, 2017. **12**(1): p. 39–42.
232. Stone, T.W. and M.N. Perkins, *Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS*. Eur J Pharmacol, 1981. **72**(4): p. 411–2.
233. Birch, P.J., C.J. Grossman, and A.G. Hayes, *Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor*. European journal of pharmacology, 1988. **154**(1): p. 85–87.
234. Huang, X.-T., et al., *An excessive increase in glutamate contributes to glucose-toxicity in β -cells via activation of pancreatic NMDA receptors in rodent diabetes*. Scientific Reports, 2017. **7**(1): p. 44120.
235. Marquard, J., et al., *Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment*. Nature Medicine, 2015. **21**(4): p. 363–372.
236. Welters, A., et al., *NMDAR antagonists for the treatment of diabetes mellitus—Current status and future directions*. Diabetes, Obesity and Metabolism, 2017. **19**: p. 95–106.
237. Huang, X.T., et al., *Activation of N-methyl-D-aspartate receptor regulates insulin sensitivity and lipid metabolism*. Theranostics, 2021. **11**(5): p. 2247–2262.
238. Petersen, J., et al., *GLP-1-directed NMDA receptor antagonism for obesity treatment*. Nature, 2024.
239. Agudelo, L.Z., et al., *Skeletal muscle PGC-1 α promotes kynurenine metabolism to increase energy efficiency and fatigue-resistance*. Nat Commun, 2019. **10**(1): p. 2767.
240. Stone, T.W., C.M. Forrest, and L.G. Darlington, *Kynurenine pathway inhibition as a therapeutic strategy for neuroprotection*. The FEBS journal, 2012. **279**(8): p. 1386–1397.
241. Lewerenz, J. and P. Maher, *Chronic glutamate toxicity in neurodegenerative diseases—what is the evidence?* Frontiers in neuroscience, 2015. **9**: p. 469.
242. Birley, S., et al., *The effects of cyclic dicarboxylic acids on spontaneous and amino acid-evoked activity of rat cortical neurones*. Br J Pharmacol, 1982. **77**(1): p. 7–12.
243. Stone, T.W. and M.N. Perkins, *Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS*. European journal of pharmacology, 1981. **72**(4): p. 411–412.
244. Prado De Carvalho, L.I.A., P. Bochet, and J. Rossier, *The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits*. Neurochemistry International, 1996. **28**(4): p. 445–452.
245. Greenwood, S.M. and C.N. Connolly, *Dendritic and mitochondrial changes during glutamate excitotoxicity*. Neuropharmacology, 2007. **53**(8): p. 891–8.
246. Ault, B. and L.M. Hildebrand, *L-glutamate activates peripheral nociceptors*. Agents and Actions, 1993. **39**(1): p. C142–C144.
247. Marvizi, J.C.G., et al., *Two N-methyl-D-aspartate receptors in rat dorsal root ganglia with different subunit composition and localization*. Journal of Comparative Neurology, 2002. **446**(4): p. 325–341.
248. Ma, Q.P. and R.J. Hargreaves, *Localization of N-methyl-D-aspartate NR2B subunits on primary sensory neurons that give rise to small-caliber sciatic nerve fibers in rats*. Neuroscience, 2000. **101**(3): p. 699–707.
249. Carlton, S.M., G.L. Hargett, and R.E. Coggeshall, *Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin*. Neuroscience Letters, 1995. **197**(1): p. 25–28.
250. Liu, H., et al., *Evidence for presynaptic N-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn*. Proceedings of the National Academy of Sciences, 1994. **91**(18): p. 8383–8387.
251. Sato, K., et al., *AMPA, KA and NMDA receptors are expressed in the rat DRG neurones*. Neuroreport, 1993. **4**(11): p. 1263–1265.
252. Lovinger, D.M. and F.F. Weight, *Glutamate induces a depolarization of adult rat dorsal root ganglion neurons that is mediated predominantly by NMDA receptors*. Neuroscience Letters, 1988. **94**(3): p. 314–320.
253. Cuesta, M.C., et al., *Opposite modulation of capsaicin-evoked substance P release by glutamate receptors*. Neurochemistry International, 1999. **35**(6): p. 471–478.
254. Marvizi, J.C.G., et al., *Neurokinin 1 Receptor Internalization in Spinal Cord Slices Induced by Dorsal Root Stimulation Is Mediated by NMDA Receptors*. The Journal of Neuroscience, 1997. **17**(21): p. 8129–8136.
255. Bardoni, R., et al., *Presynaptic NMDA receptors modulate glutamate release from primary sensory neurons in rat spinal cord dorsal horn*. J Neurosci, 2004. **24**(11): p. 2774–81.
256. Kupjetz, M., et al., *The serum kynurenine pathway metabolic profile is associated with overweight and obesity in multiple sclerosis*. Multiple Sclerosis and Related Disorders, 2023. **72**.
257. Ducommun, S., et al., *Mustn1 is a smooth muscle cell-secreted microprotein that modulates skeletal muscle extracellular matrix composition*. Mol Metab, 2024. **82**: p. 101912.
258. Nahomi, R.B., et al., *Kynurenine Acid Protects Against Ischemia/Reperfusion-Induced Retinal*

- Ganglion Cell Death in Mice. *Int J Mol Sci*, 2020. **21**(5).
259. Hayes, A.J., et al, *Kynurenine monoxygenase regulates inflammation during critical illness and recovery in experimental acute pancreatitis*. *Cell Rep*, 2023. **42**(8): p. 112763.
260. Giorgini, F., et al, *Targeted Deletion of Kynurenine 3-Monooxygenase in Mice*. *Journal of Biological Chemistry*, 2013. **288**(51): p. 36554-36566.
261. Erhardt, S., et al, *Adaptive and Behavioral Changes in Kynurenine 3-Monooxygenase Knockout Mice: Relevance to Psychotic Disorders*. *Biol Psychiatry*, 2017. **82**(10): p. 756-765.
262. Bondulich, M.K., et al, *Ablation of kynurenine 3-monooxygenase rescues plasma inflammatory cytokine levels in the R6/2 mouse model of Huntington's disease*. *Sci Rep*, 2021. **11**(1): p. 5484.
263. Ronchi, J.A., et al, *A spontaneous mutation in the nicotinamide nucleotide transhydrogenase gene of C57BL/6J mice results in mitochondrial redox abnormalities*. *Free Radic Biol Med*, 2013. **63**: p. 446-56.
264. Nicholson, A., et al, *Diet-induced Obesity in Two C57BL/6 Substrains With Intact or Mutant Nicotinamide Nucleotide Transhydrogenase (Nnt) Gene*. *Obesity*, 2010. **18**(10): p. 1902-1905.
265. Fontaine, D.A. and D.B. Davis, *Attention to Background Strain Is Essential for Metabolic Research: C57BL/6 and the International Knockout Mouse Consortium*. *Diabetes*, 2016. **65**(1): p. 25-33.
266. Simon, M.M., et al, *A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains*. *Genome Biology*, 2013. **14**(7): p. R82.
267. Jung, T.W., et al, *Administration of kynurenic acid reduces hyperlipidemia-induced inflammation and insulin resistance in skeletal muscle and adipocytes*. *Mol Cell Endocrinol*, 2020. **518**: p. 110928.
268. Lee, T., et al, *Kynurenic acid attenuates pro-inflammatory reactions in lipopolysaccharide-stimulated endothelial cells through the PPARdelta/HO-1-dependent pathway*. *Mol Cell Endocrinol*, 2019. **495**: p. 110510.
269. Zhen, D., et al, *Oral administration of kynurenic acid delays the onset of type 2 diabetes in Goto-Kakizaki rats*. *Heliyon*, 2023. **9**(7): p. e17733.
270. Wang, J., et al, *Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35*. *J Biol Chem*, 2006. **281**(31): p. 22021-22028.
271. Hillmas, C., et al, *The Brain Metabolite Kynurenic Acid Inhibits $\alpha 7$ Nicotinic Receptor Activity and Increases Non- $\alpha 7$ Nicotinic Receptor Expression: Physiopathological Implications*. *The Journal of Neuroscience*, 2001. **21**(19): p. 7463.
272. Stone, T.W., *Does kynurenic acid act on nicotinic receptors? An assessment of the evidence*. *J Neurochem*, 2020. **152**(6): p. 627-649.
273. Huang, T., et al, *Adipocyte-derived kynurenine promotes obesity and insulin resistance by activating the AhR/STAT3/IL-6 signaling*. *Nat Commun*, 2022. **13**(1): p. 3489.
274. Correia, J.C., et al, *Zfp697 is an RNA-binding protein that regulates skeletal muscle inflammation and remodeling*. *Proc Natl Acad Sci U S A*, 2024. **121**(34): p. e2319724121.
275. Mahurkar, S., et al, *Response to interferon-beta treatment in multiple sclerosis patients: a genome-wide association study*. *Pharmacogenomics J*, 2017. **17**(4): p. 312-318.
276. Emerson, R.O. and J.H. Thomas, *Adaptive evolution in zinc finger transcription factors*. *PLoS Genet*, 2009. **5**(1): p. e1000325.
277. Peterlin, B.L., et al, *Weight loss with atogepant during the preventive treatment of migraine: A pooled analysis*. *Cephalalgia*, 2024. **44**(12): p. 3331024241299753.
278. Iannone, L.F., et al, *Effectiveness of anti-CGRP monoclonal antibodies on central symptoms of migraine*. *Cephalalgia*, 2022. **42**(13): p. 1323-1330.
279. Sun, W., et al, *Adverse event reporting of four anti-Calcitonin gene-related peptide monoclonal antibodies for migraine prevention: a real-world study based on the FDA adverse event reporting system*. *Front Pharmacol*, 2023. **14**: p. 1257282.
280. Zhou, R., et al, *Calcitonin gene-related peptide promotes the expression of osteoblastic genes and activates the WNT signal transduction pathway in bone marrow stromal stem cells*. *Mol Med Rep*, 2016. **13**(6): p. 4689-96.
281. Wang, L., et al, *Calcitonin-gene-related peptide stimulates stromal cell osteogenic differentiation and inhibits RANKL induced NF-kappaB activation, osteoclastogenesis and bone resorption*. *Bone*, 2010. **46**(5): p. 1369-79.
282. Tian, G., G. Zhang, and Y.H. Tan, *Calcitonin gene-related peptide stimulates BMP-2 expression and the differentiation of human osteoblast-like cells in vitro*. *Acta Pharmacol Sin*, 2013. **34**(11): p. 1467-74.
283. Fang, Z., et al, *Effect of CGRP-adenoviral vector transduction on the osteoblastic differentiation of rat adipose-derived stem cells*. *PLoS One*, 2013. **8**(8): p. e72738.
284. Appelt, J., et al, *The neuropeptide calcitonin gene-related peptide alpha is essential for bone healing*. *EBioMedicine*, 2020. **59**: p. 102970.
285. Zhang, Y., et al, *Implant-derived magnesium induces local neuronal production of CGRP to improve bone-fracture healing in rats*. *Nat Med*, 2016. **22**(10): p. 1160-1169.
286. Yang, Q., et al, *Effect of calcitonin gene-related peptide on the neurogenesis of rat adipose-derived stem cells in vitro*. *PLoS One*, 2014. **9**(1): p. e86334.
287. Jang, J.E., et al, *Nitric Oxide Produced by Macrophages Inhibits Adipocyte Differentiation*

- and Promotes Profibrogenic Responses in Preadipocytes to Induce Adipose Tissue Fibrosis. *Diabetes*, 2016. **65**(9): p. 2516–28.
288. Chen, C.W., et al., Nitric Oxide Mobilizes Intracellular Zn(2+) via the GC/cGMP/PKG Signaling Pathway and Stimulates Adipocyte Differentiation. *Int J Mol Sci*, 2022. **23**(10).
 289. Willows, J.W., et al., Visualization and analysis of whole depot adipose tissue neural innervation. *iScience*, 2021. **24**(10): p. 103127.
 290. Walker, C.S., et al., alpha-Calcitonin gene related peptide (alpha-CGRP) mediated lipid mobilization in 3T3-L1 adipocytes. *Peptides*, 2014. **58**: p. 14–9.
 291. Linscheid, P., et al., Autocrine/paracrine role of inflammation-mediated calcitonin gene-related peptide and adrenomedullin expression in human adipose tissue. *Endocrinology*, 2005. **146**(6): p. 2699–708.
 292. Aveseh, M., et al., Serum calcitonin gene-related peptide facilitates adipose tissue lipolysis during exercise via PIPLC/IP3 pathways. *Endocrine*, 2018. **61**(3): p. 462–472.
 293. Roy, D., et al., alpha2delta1-mediated maladaptive sensory plasticity disrupts adipose tissue homeostasis following spinal cord injury. *Cell Rep Med*, 2024. **5**(5): p. 101525.
 294. Danaher, R.N., et al., Evidence that alpha-calcitonin gene-related peptide is a neurohormone that controls systemic lipid availability and utilization. *Endocrinology*, 2008. **149**(1): p. 154–60.
 295. Labastida-Ramirez, A., et al., Gender aspects of CGRP in migraine. *Cephalalgia*, 2019. **39**(3): p. 435–444.

